NOV 10 1919

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

VOLUME XXX, NO. 5 NOVEMBER 1, 1919

PUBLISHED MONTHLY
AT MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, MD.
BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

COPYRIGHT, 1919, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter June 3, 1916, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917.
Authorized June 29, 1918.

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

The Journal of Experimental Medicine is designed to cover the field of experimental medicine. Information regarding contributions and subscriptions is given in full on the back cover.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

H. D. DAKIN, New York, N. Y. LAFAYETTE B. MENDEL, New Haven, Conn.

E. K. DUNHAM, New York, N. Y. A. N. RICHARDS, Philadelphia, Pa.

DONALD D. VAN SLYKE, New York, N. Y.

The Journal of Biological Chemistry is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of between 500 and 600 pages. The subscription price is $3.00 per volume to subscribers in the United States and Canada, payable in advance; $3.25 to those in other countries. The number of volumes issued yearly averages four. The price of single copies varies; it is given on the back cover of each number. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Biological Chemistry*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF GENERAL PHYSIOLOGY

EDITED BY

JACQUES LOEB, New York, N. Y. W. J. V. OSTERHOUT, Cambridge, Mass.

The Journal of General Physiology is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter. It is issued bimonthly, one volume of about 600 pages appearing in a year. The subscription price per year (one volume) is $5.00, payable in advance. Single copies cost $1.00. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of General Physiology*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research.* The subscription price is $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine*, *The Journal of Biological Chemistry*, or *The Journal of General Physiology.* Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

SEMIANNUAL LIST

The Institute publishes a *Semiannual List* of all papers by members of the staff and those working under grants from the Institute, stating the title and place of publication of these reports. The *Semiannual List* will be sent regularly upon application.

WAVERLY PRESS
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

Chemical Series. I.

N-Phenylglycineamide-*p*-Arsonic Acid.

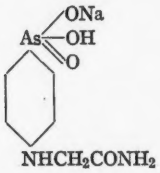
By WALTER A. JACOBS, Ph.D., and MICHAEL HEIDELBERGER, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 18, 1919.)

For a number of years the writers, in conjunction with Dr. Wade H. Brown and Dr. Louise Pearce, have been engaged in the synthesis of certain new types of organic arsenic compounds for the treatment of experimental trypanosome and spirochete infections. Although considerable of our effort has been given to the study of trivalent arsenicals our attention was at first confined to the development of compounds containing arsenic in the pentavalent form as presented by the arsonic acids. This group seemed best suited for the synthetic procedure which was employed and afforded the best opportunity for obtaining such information as to the relationship between chemical structure and biological action as would be useful for further work. Again it was felt that if a practicable and efficient drug could be found within this group it would go far toward eliminating certain of the well known practical disadvantages of the usual arseno compounds, for as an arsonic acid it should form soluble and stable salts and perhaps offer fewer of the preparative uncertainties than have been the experience with trivalent arsenicals.

In the course of these studies a number of substances have been prepared which have given interesting experimental results. However, those obtained with one in particular, the sodium salt of *N*-phenylglycineamide-*p*-arsonic acid



were such as to demand special attention. The simplicity of this compound, the ease of preparing it, its relatively inexpensive character, stability, and solubility, and its favorable biological behavior, warrant a publication of results. We wish therefore to present in conjunction with the following biological papers of Dr. Brown and Dr. Pearce, the facts as to the preparation and properties of the substance which will be appropriate in the present place. A somewhat fuller description of the substance and of related compounds has appeared elsewhere.[1]

Phenylglycine-*p*-arsonic acid, and its homologue, *o*-methylphenylglycine-*p*-arsonic acid, have already been described in German Patent, No. 204,664. These substances, of interest solely as the source of the arsenophenylglycines which were obtained by their reduction, were readily prepared by the interaction of the sodium salts of the amino-arylarsonic acids and sodium chloroacetate. In our investigations we have found that a reaction of this type, with α-halogenacylamino compounds, XCH_2CONHR, instead of the α-halogen acids, XCH_2COOH, is capable of practically unlimited extension and has rendered possible the preparation of a new series of aromatic arsenic compounds. In these substances the free carboxyl group of the above glycines has been changed to the amide or substituted amide group.

N-Phenylglycineamide-*p*-arsonic acid,[2] prepared by the methods to be described in the experimental part, readily yields a colorless,

[1] Jacobs, W. A., and Heidelberger, M., *J. Am. Chem. Soc.*, 1919, xli, 1587.

[2] This substance and related compounds to be described elsewhere are covered by United States Patents, Nos. 1,280,119–1,280,127. Patents have also been applied for in foreign countries.

All discoveries made at The Rockefeller Institute for Medical Research are made freely available to the public, in accordance with the philanthropic purposes of the institution. In order to insure purity of product and protection against exploitation, it has been deemed necessary in certain instances to protect the

crystalline sodium salt which is extremely easily soluble in water, forming neutral solutions, which are perfectly stable. In fact a 10 per cent solution may be boiled a reasonable length of time without appreciable cleavage of ammonia or arsenic.

The materials required and the method of preparation are such that the large scale production of the substance should offer little difficulty.

EXPERIMENTAL.

N-(Phenyl-4-Arsonic Acid)-Glycineamide (N-Phenylglycineamide-p-Arsonic Acid).—Of the two methods used for the preparation of this substance that described first is more direct.

434 gm. of arsanilic acid were dissolved in 2 liters of normal sodium hydroxide solution. After the addition of 375 gm. of chloroacetamide the mixture was boiled under a reflux condenser for 45 minutes, the clear solution setting to a solid mass of the crude product on cooling. 75 cc. of concentrated hydrochloric acid were added to the cold mixture to hold any unchanged arsanilic acid in solution and the substance was then filtered off and carefully washed with cold water. For purification it was suspended in sufficient water to form a thin paste and carefully treated, with stirring, with 25 per cent sodium hydroxide solution until the acid was completely dissolved. The filtered solution was then treated with an excess of acetic acid whereupon the substance separated as minute, lustrous plates. After filtering, washing thoroughly, and drying, the yield was 300 gm.

The acid is very sparingly soluble in cold water but dissolves readily on heating. It separates from the hot aqueous solution in aggregates of long, thin plates. It is insoluble in methyl alcohol, acetone, or chloroform and sparingly in hot methyl or ethyl alcohol, but dissolves in boiling acetic acid. It is sparingly soluble in dilute

discoveries by patents. It is the purpose of the Institute to permit any drugs which may prove of practical therapeutic value to be manufactured under license by suitable chemical firms and under conditions of production which will insure the biological qualities of the drugs and their marketing at reasonable prices. Other than through the issuance of license, The Rockefeller Institute does not participate in any way in the commercial preparation or sale of the manufactured chemicals; and it receives no royalties or other pecuniary benefits from the licenses it issues.

hydrochloric acid but dissolves readily in the strong acid, its behavior showing it to be a weaker base than arsanilic acid. On boiling its solution in sodium hydroxide ammonia is evolved. When rapidly heated in an open capillary tube it darkens and softens at 280°C., but does not melt.

0.1405 gm. of substance; 12.35 cc. of N (22.0°C., 761 mm.).
0.3205 gm. of substance; 0.1832 gm. of $Mg_2As_2O_7$.
Calculated for $C_8H_{11}O_4N_2As$: N, 10.22 per cent; As, 27.33 per cent.
Found: N, 10.18 per cent; As, 27.59 per cent.

Sodium Salt.—The pure acid is suspended in enough water to form a thick paste and carefully treated with 25 per cent sodium hydroxide solution until completely dissolved and the solution reacts neutral to litmus. Two volumes of alcohol are then added, the pure sodium salt quickly separating as thin, nacreous plates. After filtering and washing with 85 per cent alcohol it is air-dried and then contains one-half molecule of water of crystallization. The sodium salt is extremely soluble in cold water, the solution reacting neutral to litmus. The apparatus, filter paper, water, etc., used in the preparation of this salt must be free from calcium; otherwise a precipitate of the insoluble calcium salt will contaminate solutions of the sodium salt.

0.3921 gm. of air-dry substance, at 100°C. *in vacuo* over H_2SO_4; 0.0117 gm. loss.
Calculated for $C_8H_{10}O_4N_2AsNa.\ \frac{1}{2}\ H_2O$: H_2O, 2.95 per cent.
Found: H_2O, 2.98 per cent.
Anhydrous: 0.1503 gm. of substance; 12.45 cc. of N(25.0°C., 762 mm.).
0.2300 gm. of substance; 0.1195 gm. of $Mg_2As_2O_7$.
Calculated for $C_8H_{10}O_4N_2AsNa$: N, 9.46 per cent; As, 25.32 per cent.
Found: N, 9.52 per cent; As, 25.08 per cent.

The potassium and ammonium salts were prepared in the same way as the sodium salt and form thin, glistening, hexagonal, microscopic platelets. On adding a calcium chloride solution to a solution of the sodium salt the calcium salt gradually separates as microscopic wedge-shaped prisms, containing no water of crystallization. Magnesia mixture causes no precipitate in the cold, but on warming the magnesium salt separates as a microcrystalline powder. Heavy

metal salts give immediate precipitates, the silver salt forming aggregates of thin, microscopic needles.

N-Phenylglycineamide-*p*-arsonic acid was also prepared as follows from *N*-phenylglycine methyl ester-*p*-arsonic acid by the action of ammonia.

N-(Phenyl-4-Arsonic Acid)-Glycine Methyl Ester.—40 gm. of *N*-phenylglycine-*p*-arsonic acid[3] were treated with 120 gm. of dry methyl alcohol and 4 gm. of concentrated sulfuric acid. The mixture was boiled under a reflux condenser for 2 hours. The ester separated on cooling and scratching, the precipitation being completed by the addition of water. The filtered, washed, and dried product weighed 38 gm. It can be recrystallized from hot water or hot 95 per cent alcohol, separating from the former as microscopic needles and thin plates. It is very sparingly soluble in cold water, cold alcohol, or boiling acetone, and is fairly easily soluble in methyl alcohol, especially on warming. When rapidly heated it softens and darkens above 200°C. and decomposes at about 285°.

0.1560 gm. of substance; 7.0 cc. of N (21.0°C., 747 mm.).
0.3135 gm. of substance; 0.1665 gm. of $Mg_2As_2O_7$.
Calculated for $C_9H_{12}O_5NAs$: N, 4.84 per cent; As, 25.94 per cent.
Found: N, 5.12 per cent; As, 25.63 per cent.

The ester was converted into the amide as follows: 10 gm. of the ester were slowly added, with stirring, to 30 cc. of well chilled, concentrated ammonia. At first a thick paste of the ammonium salt of the ester was formed, but on allowing the mixture to rise to room temperature the reaction proceeded with formation of a clear solution. After 24 hours the excess of ammonia was removed, preferably *in vacuo*. On diluting with water, filtering, and acidifying with acetic acid, *N*-phenylglycineamide-*p*-arsonic acid separated in characteristic form. This was purified as described above and was identical in every way with the product obtained by the direct method. The yield was 80 per cent of the theory.

0.1297 gm. of substance; 11.5 cc. of N (19.5°C., 742 mm.).
Calculated for $C_8H_{11}O_4N_2As$: N, 10.22 per cent.
Found: N, 10.11 per cent.

---

[3] German Patent, No. 204,664.

CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

Biological Series. I.

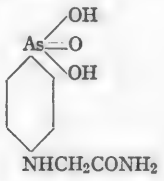
The Toxic Action of N-Phenylglycineamide-*p*-Arsonic Acid.

By WADE H. BROWN, M.D., and LOUISE PEARCE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 18, 1919.)

The chemotherapeutic investigations which have been in progress in these laboratories for several years have yielded a number of substances of striking activity in the treatment of experimental infections such as those produced in laboratory animals by various species of trypanosomes, the spirochetes of relapsing fever, and *Treponema pallidum*. Among the first of these substances was the amide of *N*-phenylglycine-*p*-arsonic acid, a description of which is given by Dr. Jacobs and Dr. Heidelberger, in the chemical series of these papers.[1] It may be said that while *N*-phenylglycine-*p*-arsonic acid, described in German Patent, No. 204,664, is a substance of practically no importance in the treatment of these infections, arsenophenylglycine, produced from it by reduction, was among the earliest of the highly active trypanocidal agents. The amide of this acid which has the structural formula



was first made and studied in the fall of 1915 with results which at once opened the way to the development of a number of important substances which will be dealt with in subsequent papers.

[1] Jacobs, W. A., and Heidelberger, M., *J. Exp. Med.*, 1919, xxx, 411.

Methods of Employing N-Phenylglycineamide-p-Arsonic Acid.

The amide of *N*-phenylglycine-*p*-arsonic acid, or A 63 as it was designated on our lists, presents no difficulty in the way of its use for purposes of animal experimentation. In the form of the monosodium salt, the drug is readily soluble in water in concentrations as high as 50 per cent and when injected directly into the tissues or into the body cavities of animals, it is readily absorbed and produces but slight irritation or local injury.

Preparation of Solutions.—Solutions of A 63 may be prepared by dissolving the monosodium salt in sterile distilled water, or in case the acid is used, the requisite amount of sodium hydroxide (0.37 cc. of N sodium hydroxide per 0.100 gm. of drug) to form the monosodium salt should be added slowly with stirring. The product then dissolves without difficulty.

Administration.—This drug may be administered to animals by almost any route which is convenient and has been used subcutaneously, intramuscularly, intraperitoneally, intravenously, and *per os* with no especial disadvantage following its use by any except the last named route.

Measurement of Doses.—The measurement of doses was accomplished in one of three ways. With the smaller animals, a stock solution of the drug was prepared from which individual doses for mice were measured by the use of a Fornier tuberculin syringe graduated in hundredths of a cubic centimeter; in the case of rats and guinea pigs, doses were measured with standardized pipettes. Finally, doses for larger animals such as rabbits and monkeys were always weighed and prepared separately. The values given in all cases refer to amounts of the monosodium salt which was used almost exclusively. These figures may be transposed into equivalents of the acid by the use of the factor 0.9 if desired.

Minimum Lethal Dose.

Although A 63 contains 24.57 per cent of arsenic, its toxicity for laboratory animals is comparatively low. The lethal dose of the drug varies between the extremes of 0.75 and 2.75 gm. per kilo of body weight for the different animal species in which it has been tested and for different routes of administration.

Mice.—The tolerance of mice is particularly good, as is indicated by the figures given in Table I which represents the combined results of several experiments carried out with mice. From the data at our disposal, we would place the minimum lethal dose for mice at 2.5 to 2.75 gm. per kilo of mouse when given subcutaneously, 2 to 2.25 gm. given intraperitoneally, and 2 gm. when given intravenously. These figures show a reasonably close agreement in the toxicity of the drug when administered by these routes, and the results obtained from successive experiments were comparatively uniform. Some mice survive even higher doses than those shown in the table, and conversely an occasional mouse may succumb to lower doses than those given as the lethal or minimum lethal dose; but we have had no

TABLE I.

Lethal Effects Obtained from the Administration of a 5 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Mice in Doses Equivalent to 1.75 to 2.5 Gm. per Kilo of Mouse.

Dose per kilo.	Subcutaneous injection.		Intraperitoneal injection.		Intravenous injection.	
	No. of mice used.	No. died.	No. of mice used.	No. died.	No. of mice used.	No. died.
gm.						
2.5	2	0	5	1	2	2
2.25	4	0	21	5	4	1
2.0	4	0	25	2	4	1
1.75	4	0	8	0	4	0

deaths resulting from doses of the drug below 2 gm. per kilo of mouse.

Rats.—The resistance of white rats to the toxic action is lower and more irregular than that of any other animal with which we have worked. When the drug is administered intraperitoneally, doses as small as 0.75 gm. per kilo are sufficient to cause death in a fair percentage of animals and yet some rats will survive as much as 1.75 gm. per kilo. The results obtained from subcutaneous administration are distinctly better; the minimum lethal dose rises to 1 gm. per kilo and the action of the drug is definitely more constant, as may be seen by an examination of the results from the two experiments incorporated in Table II.

TABLE II.

Lethal Effects Obtained from the Subcutaneous Administration of a 10 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Rats and from the Intraperitoneal Administration of a 3 Per Cent Solution.

Dose per kilo.	Subcutaneous injection.		Intraperitoneal injection.	
	No. of rats used.	No. died.	No. of rats used.	No. died.
gm.				
1.50	4	4	5	4
1.25	4	1	5	3
1.00	9	6	9	7
0.90	5	0	5	2
0.75	5	0	5	3
0.60			5	0
0.50			5	0

Guinea Pigs.—Contrary to the opinion which is generally held as to the tolerance of guinea pigs for arsenicals, these animals withstand relatively large doses of A 63 and the resistance of individual animals appears to be fairly uniform. The lethal dose found for the drug was 1.5 gm. per kilo of body weight whether given subcutaneously or intraperitoneally (Table III).

TABLE III.

Lethal Effects Obtained from the Administration of a 20 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Guinea Pigs.

Dose per kilo.	Subcutaneous injection.		Intraperitoneal injection.	
	No. of guinea pigs used.	No. died.	No. of guinea pigs used.	No. died.
gm.				
1.75			4	1
1.50	4	3	9	3
1.40	4	0	4	0
1.25	4	0	4	0

Rabbits.—A great deal of time has been devoted to the study of the toxic action of A 63 in rabbits. The major part of this work was carried out by intravenous administration of the drug but the effects

of subcutaneous, intramuscular, and *per os* administrations have all been investigated to some extent. The solutions used in the experiments varied between concentrations of 5 and 50 per cent, partly for the purpose of studying the effects of the use of different volume doses and partly for the purpose of determining the influence of such factors as rate of administration and concentration of solutions upon the action of the drug.

It was found that as much as 20 cc. per kilo of a 5 to 10 per cent solution could be injected intravenously into rabbits about as rapidly as one wished, and that in general, solutions of low concentration (5 to 10 per cent) were better borne than those of a higher concentration. The effects of concentration were less evident, however, when the drug was administered by other routes, and more concentrated solutions were employed as a means of reducing the volume of fluid which had to be used.

Under the conditions described, the minimum lethal dose for rabbits was found to be 0.75 to 0.9 gm. per kilo of body weight when given intravenously and 1.1 gm. given either subcutaneously or intramuscularly, with lethal effects as indicated by the experiments recorded in Table IV.

TABLE IV.

Lethal Effects Obtained from the Intravenous Administration of a 5 to 10 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Rabbits and from the Subcutaneous or Intramuscular Administration of 50 Per Cent Solutions.

Dose per kilo.	Subcutaneous injection.		Intramuscular injection.		Intravenous injection.	
	No. of rabbits used.	No. died.	No. of rabbits used.	No. died.	No. of rabbits used.	No. died.
gm.						
1.25	8	6	4	3		
1.1	4	4	4	2		
1.0	3	0	3	0	6	3
0.9					12	4
0.75					10	1

Our experience in giving this drug to rabbits by mouth may be recited very briefly. The drug was given in solution by means of

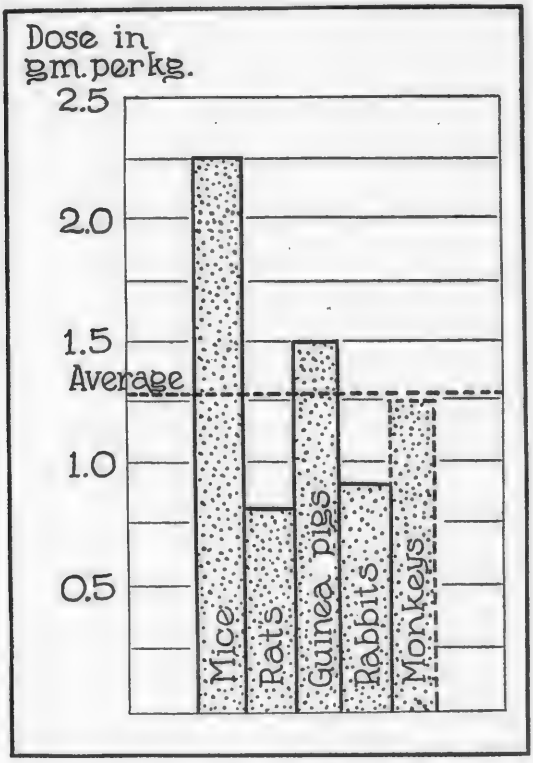
a stomach tube following the administration of a small dose of bicarbonate of soda. In all, there were only seven rabbits which received the drug in this way. Two of them were given doses of 1.25 gm. per kilo of body weight, four were given 1 gm., and one was given 0.75 gm. The last of these animals showed no ill effects from the drug and only two of the others actually succumbed to its action, both of which received a dose of 1 gm. per kilo. One of these rabbits lived 4 days and the other 24 days after the administration of the drug. It appeared certain, however, that none of the other animals would recover completely and they were killed at different times for purposes of pathological examination. As the matter stands, therefore, we are hardly justified in attempting to fix the toxic limits of A 63 when given *per os*.

Monkeys.—Lastly, we have used two monkeys (*Macacus rhesus*) in studying the toxic action of A 63. The first monkey, a female weighing 2,325 gm., was given an initial dose of 0.75 gm. per kilo of body weight. The animal was observed for 5 days during which time no ill effect from the drug could be detected, and a second dose of 1 gm. per kilo was given with a like result. 4 days later, the animal received its third dose which was 1.25 gm. per kilo. Following this dose, the only evidence of intoxication noted was possibly a slight loss of weight (75 gm.). At the end of 3 weeks, the weight of the animal was 2,450 gm., and a fourth dose of the drug was given—this time 1.5 gm. per kilo, or double the initial dose. The monkey showed slight signs of intoxication lasting for a few days, followed by rapid recovery. The animal was kept under observation for 3 months and was then killed for pathological purposes.

The second monkey used was likewise a female *rhesus* weighing 2,650 gm. This monkey was given an initial dose of 1.25 gm. per kilo intravenously. There was a loss of weight in this animal amounting to 175 gm., but no other evidence of intoxication developed, and after waiting 1 week, a second dose of 1.5 gm. per kilo was given; death followed within 24 hours.

These two experiments are cited merely to show that monkeys possess a degree of tolerance for A 63 comparable with that of other animals and that they react to the drug in much the same way.

Represented graphically, the values obtained for the minimum lethal dose of this drug in the five species of animals studied would form a curve such as that given in Text-fig. 1. While these values are not strictly comparable on account of differences in the mode of



TEXT-FIG. 1. Comparative magnitudes of the minimum lethal dose of *N*-phenylglycineamide-*p*-arsonic acid for different animal species. The dose given for monkeys is an approximate estimate.

administration used, they will serve to indicate the relative magnitude of the toxic dose for different animal species. The mean toxic dose for this group of animals is found to be approximately 1.28 gm. per kilo; the maximum variation from this mean is as 1: 1.75 and the extremes compare as 1: 2.8.

Symptoms and Course of the Intoxication.

The symptom-complex of the intoxication produced by *N*-phenylglycineamide-*p*-arsonic acid in laboratory animals is characterized by two groups of phenomena, one nervous and the other nutritional in character. The most prominent of these symptoms appear at an early period of the intoxication, as pronounced tremors with incoordination of movements, or in extreme cases, as clonic spasm, usually associated with some weakness or complete prostration. With remission of these early symptoms in mice, a tic develops which is characterized by peculiar jerky movements of the head and occasionally by the continuous circling movements of dancing mice. Nutritional disturbances are indicated chiefly by a loss of appetite, more or less weakness, loss of weight, and occasionally by diarrhea. Altogether the picture is one previously recognized and described as characteristic of the toxic action of a number of pentavalent arsenicals, particularly of arsacetin, dichlorophenolarsonic acid, and aminohydroxyphenylarsonic acid.

These symptoms are by no means constant either as to the frequency or the intensity of their occurrence in different animal species or in individual animals of the same species.

Mice.—In mice symptoms of intoxication occur only following relatively large doses of the drug (1.75 gm. per kilo and above) and even then are not of constant occurrence. In the most marked cases, mice show violent muscular tremors on being disturbed and marked locomotor incoordination. After 2 or 3 days, these symptoms disappear entirely or gradually give place to the tic described, which in turn rarely persists for more than a week. Disturbances of this general character appear to be more pronounced following subcutaneous and intravenous administrations of the drug than after intraperitoneal administration.

Mice also show some nutritional disturbances for 2 or 3 days following toxic doses of the drug but make a rapid recovery with an increase in weight above their normal level and remain subsequently in excellent condition.

Rats.—Rats are, on the whole, the most sensitive animals to this drug and individual idiosyncrasy is quite pronounced among them.

Nevertheless, toxic symptoms in these animals are fairly well confined to doses within what we regard as the lethal range. While some rats show no toxic symptoms at all, in others, tremors, incoordination, convulsions, weakness, and emaciation reach a most extreme grade and progress to a lethal termination, recovery under such conditions being very rare.

Guinea Pigs.—Guinea pigs on the other hand are quite resistant to A 63. They show very slight evidence of intoxication either of a nervous or of a nutritional character from doses below the lethal level. The dose that is survived is usually well borne and even lethal doses produce comparatively slight tremors, unsteadiness, weakness, or loss of weight.

Rabbits.—The reaction of rabbits to A 63 is rather irregular. By whatever route the drug is administered, doses above 0.5 to 0.6 gm. per kilo of body weight cause some loss of appetite with an initial loss of weight which is in proportion to the size of the dose used. With doses of 0.75 to 0.9 gm. per kilo of body weight, the loss of weight in surviving animals may reach as much as 200 to 300 gm. and is associated with some degree of weakness. This loss of weight, however, is quickly regained, leaving no symptomatic evidence of intoxication. Nervous phenomena of the exact type described in mice and rats do not occur in rabbits. Instead, rabbits manifest some hypersensitiveness in the milder cases of intoxication, while in extreme cases incoordination develops with a tendency to clonic spasms, and there is eventual loss of muscular control or even paralysis. As in the case of the rat, pronounced nervous symptoms in rabbits nearly always portend a lethal outcome so that these phenomena cannot be said to occur as symptoms of a sublethal intoxication.

When toxic doses are given to rabbits *per os*, the drug produces symptoms which are not observed when it is given by other routes, the characteristic feature of which is a marked abdominal distention with flatulence as a result of atony of the colon. Associated with this condition, there is a loss of appetite, diarrhea, progressive loss of weight, and weakness. The condition is extremely persistent and recovery, if it occurs at all, is problematical.

Monkeys.—In our limited experience with monkeys, we have observed no symptoms of intoxication other than slight loss of weight and some weakness as previously recorded.

Having described the symptoms of intoxication produced by A 63, we shall now take up the question of the time element in these toxic reactions. In mice symptoms of intoxication are usually apparent within 24 hours after the administration of the drug but may be delayed until the 2nd day. Death also occurs relatively early in these animals and is rare after 48 to 72 hours. Symptomatic recovery is relatively prompt.

Rats, on the other hand, usually show no symptoms of intoxication until the 2nd or 3rd day except with very large doses of the drug. Out of twenty-eight rats that died as a result of intoxication with doses below 1.5 gm. per kilo of body weight, the earliest death occurred 3 days after the administration of the drug, the latest deaths 10, 11, and 14 days, while the average period was 6 days. Recovery in rats is also a slow and uncertain process.

Symptoms of intoxication in guinea pigs usually make their appearance on the 2nd day following the administration of the drug. Among the deaths recorded in our series from doses below 2 gm. per kilo, the earliest occurred 2 days and the latest 7 days after the administration of the drug with an average period of 4 days survival. Recovery in guinea pigs is usually prompt and complete.

Of the rabbits which died after intravenous injections of a single dose of A 63, one with the smallest dose (0.75 gm.) died in almost exactly 24 hours, while the longest survival was 6 days and the average 2.6 days. The minor symptoms of intoxication in rabbits are not apparent as a rule until the 2nd day, while the graver symptoms occur more promptly. As previously indicated, symptomatic recovery in the rabbit is usually prompt and complete.

Pathology of the Intoxication.

The pathological changes produced in the animal organism by toxic doses of A 63 constitute an important phase of its toxicologic action. Viewed from the standpoint of the bearing of these changes upon the possible usefulness of the drug as a therapeutic agent, this phase of the subject resolves itself largely into a consideration of organic injury and recovery therefrom.

Local Effects.

As we have already indicated, the injury produced by the drug at the site of injection is almost negligible. Full toxic doses may be injected into the veins of animals even in saturated solution, with practically no local reaction. When a 50 per cent solution of the drug is injected into the subcutaneous tissues of animals, a slight edema develops at the site of the injection but clears up almost immediately, leaving such slight evidences of tissue reaction as are barely recognized from external examination. If given intramuscularly in amounts small enough to obviate mechanical laceration of the tissues, the reaction which follows is likewise very mild and is of essentially the same character as that which follows subcutaneous administration.

Systemic Effects.

The effects produced by the drug upon the organism as a whole are of much more importance than those of a purely local character. These effects are divisible into major and minor phases of pathological action, the details of which differ somewhat in different animal species as well as with the amount of the drug used and the route of administration employed. In general, however, the changes seen during the early stages of the intoxication consist in moderate vascular dilatation and congestion with a few scattered petechial hemorrhages, occasional effusions into the serous cavities, and widespread cellular degenerations or even necrosis in some organs. The organs which share most prominently in these changes are the kidneys and adrenals, and the cardiovascular system, with an uncertain involvement of the central nervous system, the gastrointestinal tract, the blood, and blood-formative organs; the changes produced in other organs appear to be of minor importance. Animals which survive the intoxication rarely show lesions in organs other than the kidneys and myocardium.

Kidneys.—The central feature of the pathological action in all animals is the injury to the kidneys. In acute poisoning, the kidneys are somewhat enlarged and tend to be pale except for the presence of more or less congestion or even hemorrhage in the boundary zone

which may extend outward along the medullary rays to the capsular surface. The glomerular tufts are usually swollen, the capillaries congested, and the covering epithelium is degenerated. Degeneration or even necrosis of the tubular epithelium is rather widespread but occurs chiefly in the ascending loops of Henle and the convoluted tubules, particularly those located along the medullary rays and the outer portion of the cortex. The interstitial elements of the kidney are, as a rule, much less affected except when overwhelming doses of the drug are given.

As serious as these injuries appear to be in the acute stages, recovery is remarkably satisfactory in most animals. Reaction of the tissue elements sets in promptly and restoration of the injured parts is accomplished within a short period of time and with comparatively slight scarring or distortion of the architecture except where the initial destruction was unusually extensive. In these cases, the marks of injury are found in a growth of connective tissue and in round celled infiltrations extending along the medullary rays and through the outer portion of the cortex, while a few glomeruli show shrunken tufts and thickened capsules, all of which gives a granular surface to the kidneys.

Adrenals.—Evidences of injury to the adrenals consist in swelling, with some congestion of the cortical vessels and the occasional presence of focal hemorrhages in the cortex. In guinea pigs, the later changes are manifested by a decrease in the cortical pigmentation with well marked degenerative changes in the cortex and medulla or even necroses in the midcortical zone. Here again recovery appears to take place fairly promptly.

Cardiovascular System.—The most pronounced effects of A 63 upon the cardiovascular system appear during the early stages of the intoxication. There is a moderate vascular dilatation and congestion throughout the body and a few petechial hemorrhages may occur in any of the organs. The sites of chief importance are the kidneys, the adrenals, and heart in which occasional foci of hemorrhage are found beneath the epicardium and the endocardium as well as in the myocardium itself. In several instances, hemorrhages from the meningeal vessels have been observed, either in the region of the torcular or in the retroorbital tissues.

Animals which survive the acute poisoning show little evidence of either congestion or hemorrhage, but in their stead one finds an accumulation of more or less fluid in the serous cavities, perirenal edema, and in rare instances an increased tension of the cerebrospinal fluid. These conditions we regard as at least indicative of vascular injury. In addition to such changes as these, the myocardium shows a moderate fatty degeneration with occasional areas of fibrosis. On the whole, the pathological effects of A 63 upon the cardiovascular system are not pronounced and the conspicuous congestion, hemorrhage, and degeneration so often observed with arsenicals are largely absent from the changes which characterize the action of this drug.

Central Nervous System.—From the symptoms of the toxic action of A 63, one might expect the central nervous system to share the position of chief pathological importance with the kidneys and cardiovascular system. Such changes as occur, however, are rather obscure and difficult of determination in laboratory animals. We have already mentioned the occurrence of congestion of the meningeal vessels and occasional hemorrhages together with an increased tension of the cerebrospinal fluid. Histologically, the chief evidences of injury are associated with the choroid plexus and with the small and medium vessels of the meninges and brain. The plexus itself shows definite degeneration in some animals and the tissues immediately surrounding the vessels are somewhat edematous. Apart from these changes, however, we have been able to demonstrate only minor degrees of cellular degeneration of a rather indefinite character and of uncertain extent.

Blood and Blood-Formative Organs.—While the action of A 63 upon the blood and blood-formative organs forms an important feature of the effect of the drug, this action is perhaps more physiological than pathological and hence there is little that can be said in the present connection.

Poisoning with the drug produces an initial destruction of red blood cells and an accumulation of blood pigments in the spleen, liver, and bone marrow. The formative centers, especially those of the spleen, show degeneration or even necrosis after the administration of very large doses of the drug, but following these changes, there is an extremely active hyperplasia in which all elements participate.

This is most strikingly shown by a myeloid metaplasia in the spleen of rats and mice which is characterized by the presence of large numbers of megacaryocytes. These changes alone, however, might mean very little, since such reactions are easily excited in mice and rats.

The effect of A 63 upon other organs of the body is relatively inconsiderable. In the liver, parenchymatous and fatty degeneration are usually but not constantly present, and in exceptional instances isolated cells or groups of cells here and there show necrosis. As a rule, these changes clear up very quickly leaving nothing to indicate the existence of any previous injury.

Lesions of the gastrointestinal tract are likewise inconstant and of uncertain significance. During the early stages of the intoxication, a catarrhal condition of the entire tract is not infrequently observed but is rarely of serious degree. An exception to this rule is found in animals which have been given the drug by mouth. These animals always show a pronounced and persistent colitis with a lesser degree of involvement of the stomach and small intestine. This, however, is clearly referable to the mode of administration of the drug.

Apart from a knowledge of the character of the lesions which may be induced by this drug, the features of chief interest from the pathological point of view are the promptness with which repair is accomplished and the fact that, as the dose of the drug used falls below the level of the lethal dose, the extent of the resulting injury rapidly diminishes until, with doses only slightly below the level of the lethal dose, organic injury is barely demonstrable. These features of the pathological action of A 63 are both unusual and of considerable practical importance in their bearing upon the use of the drug for therapeutic purposes.

Tolerance of Repeated Doses.

The facts presented thus far have dealt entirely with effects produced by the administration of single doses of the drug, which brings us to a consideration of the reaction of the animal organism to the use of repeated large doses. This phase of drug action is bound up to a large extent with two conditions, first, the time during which the drug remains in the animal body in a form capable of exercising a

toxic effect, and second, the character and duration of the effect produced, whether functional or organic.

In attempting to arrive at some idea of the time during which A 63 remained biologically active after being administered to animals, we made use of two types of experiments—one based upon protection against infection and the other upon superposition of fractional parts of toxic doses. In general, it was found by experiments of the first class, that the protection afforded such animals as mice against infection with *Trypanosoma brucei* was practically nil 24 hours after the administration of as much as twice the dose of the drug which was capable of curing a 24 hour infection of the same organism (0.5 gm. per kilo). With rats and with rabbits on the other hand, some degree of protection still existed at the end of 48 hours. In the case of the rabbit, four animals which were inoculated with *Trypanosoma brucei*, 48 hours after having received an intravenous injection of 0.5 gm. of A 63 per kilo, gave the following results: One rabbit showed no protection, two were permanently protected, and the fourth showed an incubation period of 21 days as contrasted with an incubation period of 9 days in controls which received the same dose of organisms at the same time.

From these experiments it appeared that retention of the drug in a biologically active form was a variable condition both specifically and individually, but that in animals such as the rat and the rabbit, there was the possibility of the drug's remaining in the body for 48 hours or even longer in a condition in which it might still be capable of exerting toxic effects. To test this further, we carried out a few experiments on the effects of the administration of fractional parts of a toxic dose at various intervals of time. Without going into the details of these experiments, we may say that it was found that when the toxic dose of A 63 for rabbits (0.75 gm. per kilo) was divided into three equal parts administered at intervals of 24 hours, the toxic effect approached that produced by the administration of the entire amount of drug at one time. This, of course, might be interpreted either as evidence of an accumulation of drug or as evidence of superposition of effects notwithstanding the fact that no toxic effect could be recognized from the administration of a single such fractional part of the toxic dose.

Combining these facts with what we had already learned in regard to the general reaction of animals to single doses of A 63, we undertook some experiments intended to determine the tolerance of mice and of rabbits to prolonged repetition of large doses of the drug and something of the interval at which such repetitions might be carried out successfully. The results obtained with one series of mice are summarized in Table V.

These mice were given an initial dose of 2 gm. of A 63 per kilo of body weight injected into the peritoneal cavity. The injections were repeated at weekly intervals and the dose was progressively increased up to 3 gm. per kilo. The sixth and last dose of 2.5 gm. per kilo was

TABLE V.

Tolerance of Mice to Intraperitoneal Administrations of Increasing Doses of N-Phenylglycineamide-p-Arsonic Acid Given at Weekly Intervals.

No. of injection.	Dose per kilo.	No. of mice used.	No. intoxicated.	No. died.
	gm.			
1	2.00	15	5	2
2	2.25	13	0	0
3	2.50	13	2	2*
4	2.75	11	0	0
5	3.00	11	0	0
6†	2.50 (i.v.)	9	3	3

* These two mice were toxic after the first dose.

† The last injection was given intravenously.

then given intravenously. Five of the fifteen mice showed symptoms of intoxication following the administration of the first dose and two of these died. Two others died after receiving the third dose (2.5 gm.) but the remaining eleven mice survived the administration of 3 gm. per kilo with no evidence of intoxication, indicating a definite increase in their tolerance to intraperitoneal administrations of the drug. When the route of administration was changed, these animals still showed a resistance to the drug slightly greater than that of normal mice.

Experiments of a similar character were carried out with rabbits. In this case, three routes of administration were used—subcutaneous, intramuscular, and intravenous. The initial doses were placed at

what was regarded as comparable levels for the various routes of administration; *i.e.*, 0.75 gm. per kilo intravenously and 1 gm. per kilo given either subcutaneously or intramuscularly. Animals of the subcutaneous and intramuscular series received their last dose intravenously and those of the intravenous series were divided into two

TABLE VI.

Tolerance of Rabbits to Increasing Doses of N-Phenylglycineamide-p-Arsonic Acid Given at Weekly Intervals, (A) Subcutaneously, (B) Intramuscularly, and (C) Intravenously.

No. of injection.	Dose per kilo.	No. of rabbits used.	No. intoxicated.	No. died.
	gm.			
A 1	1.00	3	0	0
2	1.00	3	0	0
3	1.10	3	0	0
4	1.25	3	0	0
5	1.35	3	0	0
6*	1.10	3	0	0
B 1	1.00	3	0	0
2	1.00	3	0	0
3	1.10	3	0	0
4	1.25	3	0	0
5	1.35	3	0	0
6*	1.10	3	0	0
C 1	0.75	10	2	1
2	0.75	9	1	0
3	0.90	9	0	0
4	1.00	9	1	1
5	1.10	8	0	0
6*	1.35	8	1	1

* The last injections of Series A and B were given intravenously; in Series C, four rabbits were injected subcutaneously and four intramuscularly.

groups one of which was given the last dose subcutaneously and the other intramuscularly. The results of these experiments are given in Table VI.

Following the first dose, one rabbit of the intravenous series was extremely toxic and died within 24 hours. A second rabbit was slightly toxic but all the others remained in good condition. No

other toxic manifestations developed until the fourth dose of the series was given, when one rabbit of the nine which received the dose of 1 gm. per kilo intravenously became toxic and died after 17 days. The fourteen remaining rabbits were carried through to the conclusion of the experiment with no symptoms suggestive of a harmful effect other than slight fluctuations in weight.

The dose in the intravenous series was raised progressively from 0.75 gm. per kilo of body weight to 1.1 gm., which in our experience is almost uniformly fatal when given to normal rabbits, with the loss of only two out of the ten rabbits; the eight rabbits which received the dose of 1.1 gm. survived with no evidence whatsoever of intoxication. These eight rabbits were then given a dose of 1.35 gm. of A 63 per kilo of body weight either subcutaneously or intramuscularly with one death following an intramuscular injection. The other rabbits remained entirely normal and continued to gain weight. With the subcutaneous and intramuscular series, the dose was raised from 1 gm. per kilo of body weight to 1.35 gm. with no toxic developments, and all these animals were then given 1.1 gm. of the drug by the intravenous route. In this case, the weights declined slightly but there was no other sign of intoxication. It is to be noted that all three groups of rabbits were carried to a point where they not only survived doses which are ordinarily fatal but that when a normally lethal dose was given by a route other than that to which they had been accustomed, only one of the fourteen rabbits succumbed, while the others showed little or no ill effects.

At the conclusion of this experiment, the entire group of rabbits was killed for pathological examination. On opening these animals, there was a strong odor of garlic—a condition never noted in an animal which had died from a single dose of the drug. Pathologically, the only changes found were small subcutaneous abscesses or abscesses in the lumbar muscles of animals which had received repeated injections in the same regions, a slight fatty degeneration of the myocardium with a few patches of fibrosis, and chronic nephritis. The injuries to the heart muscle and to the kidneys were definitely more pronounced in the rabbits of the intravenous group than in the others. With reference to the kidneys of this group, we would classify them as two with slight lesions, two with moderate lesions, and four with pronounced lesions.

The total amount of drug received by each of these rabbits during a period of 36 days was 5.85 gm. per kilo in one group and 6.8 gm. per kilo in the other, or 1.437 to 1.67 gm. of arsenic administered within a period of 5 weeks. Finally, it should be recalled that the toxic dose of A 63 for rabbits is lower than that for any other animal with which we have worked except the rat, and that the mice previously referred to received more than twice these amounts of the drug.

Our limited experience with monkeys, as previously stated, merely served to indicate that under properly chosen conditions, they too would respond to repeated doses in essentially the same way as rabbits or mice. While we do not know what the lethal dose of this drug is for monkeys, it is probably not less than 1 to 1.25 gm. per kilo of body weight, or approximately the same as that for guinea pigs and considerably more than that for the rabbit. In the one instance in which gradually increasing doses of A 63 were given to a monkey, we succeeded in giving as much as 1.5 gm. per kilo as the final dose with very slight intoxication resulting. When this animal was killed 3 months later for pathological examination, the organs appeared normal.

CONCLUSION.

The essential facts to be gathered from these studies of the toxicologic action of *N*-phenylglycineamide-*p*-arsonic acid may be summarized very briefly. The substance is one which lends itself well to almost any method of administration and can be given to animals in very large doses. The tolerance of different animal species varies rather widely but with one exception the reaction of laboratory animals to toxic doses of the drug is of favorable character. That is, toxic effects are confined to doses relatively close to the minimum lethal dose and the recovery of animals from sublethal intoxications is remarkably rapid and complete. This feature of the action of the drug makes possible the repeated administration of even very large doses at comparatively short intervals of time without incurring the dangers incident to cumulative action or to superposition of toxic effects. On the contrary, by taking advantage of this peculiarity of action, it is possible to develop such a degree of tolerance on the part of animals that the dose of the drug administered can be progressively

increased to a point well above that which is fatal to the normal animal, and this stands out as the feature of the toxicologic action of *N*-phenylglycineamide-*p*-arsonic acid which is of greatest significance in the use of the drug for therapeutic purposes.

CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

Biological Series. II.

The Therapeutic Action of N-Phenylglycineamide-*p*-Arsonic Acid in Experimental Trypanosomiasis of Mice, Rats, and Guinea Pigs.

By LOUISE PEARCE, M.D., and WADE H. BROWN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 18, 1919.)

The arsenical compound *N*-phenylglycineamide-*p*-arsonic acid, the toxic action of which has been described in the preceding paper, exercises a powerful therapeutic effect in experimental trypanosomiasis of laboratory animals. Trypanosomiasis as it occurs in mice and rats is chiefly characterized by the constant and progressively increasing number of trypanosomes in the peripheral blood stream, by the usual lack of any clinical manifestations, and by the relatively early death of the infected animal. An effective therapeutic compound, therefore, for the treatment of trypanosomiasis of mice and rats must be biologically available within a very short time after its administration and must have a sufficient speed and duration of action to halt and eventually overcome a rapidly increasing blood infection comparable, in part, with a bacteriemia, which if not checked, will cause the death of the animal in a few days or even hours. On the other hand, trypanosomiasis of many of the larger animals, especially of the rabbit, is preeminently a tissue infection and is characterized, in the acute stages, by conspicuous edematous and inflammatory swellings of the soft parts, particularly of the head and external genitalia, together with loss of appetite, weakness, and emaciation, while in the more chronic phases of the infection, the inflammatory lesions undergo induration and even necrosis with involvement of the deeper tissues including the periosteum and bone. The duration of the infection in

rabbits is a matter of weeks or months and the presence or absence of trypanosomes in the blood stream is distinctly of minor importance. It is obvious that a drug which is used for the treatment of this type of infection must possess in addition to trypanocidal action, the power of penetrating diseased tissue, and since this may require a considerable amount of time, the drug in question must remain biologically active in the animal host as long as may be necessary.

In order, therefore, to arrive at a full appreciation of the therapeutic action of a drug in experimental trypanosomiasis, one has, from the point of view of the animal factor, two general types of infection, the treatment of which will furnish information as to the speed of action on the one hand and as to the duration of action or potency of the drug on the other. This information, which should include such data as the determination of a therapeutic range in different animal species as well as curative doses, the therapeutic action against different species of trypanosomes, the comparative value of different routes of administration, and the results of a repeated dose system of therapy, together with the data upon the toxicological and pathological action of the drug in question in various animal species, furnishes a logical foundation for an accurate appraisal of the action of the drug under experimental laboratory conditions and, at the same time, furnishes a basis for estimating its probable value in the treatment of trypanosomal infections as they occur in nature.

The present paper includes the therapeutic results obtained with the amide of *N*-phenylglycine-*p*-arsonic acid in experimental trypanosomiasis of mice, rats, and guinea pigs. While trypanosomiasis of guinea pigs is, strictly speaking, a chronic infection of cyclic character, the results of its treatment with this drug are incorporated with those of acute infections of mice and rats.

EXPERIMENTAL.

Strains of *Tr. brucei*, *Tr. gambiense*, *Tr. equinum*, *Tr. equiperdum*, and *Tr. evansi*, carried constantly in stock mice and stock guinea pigs, were used for the infecting organisms, and all inoculations were made intraperitoneally. The details of animal inoculation for therapeutic purposes and the general conduct of such experiments have been de-

scribed[1] and need not be repeated here; but in this connection, mention may be made of the desirability of regulating and maintaining a uniform grade of infection, a factor of importance in interpreting therapeutic results. In the majority of experiments, we used a strain of *Tr. brucei* which is highly virulent and uniformly fatal for mice, rats, and guinea pigs, mice dying in from 60 to 70 hours, rats in from 4 to 5 days, and guinea pigs on an average of 4 weeks after the usual intraperitoneal inoculation. With organisms of less virulence, the size of the inoculating dose was proportionately adjusted to insure an infection comparable with that produced by our strain of *Tr. brucei*.[2] Both mice and rats were treated 18 to 24 hours and guinea pigs usually 1 week after inoculation, after parasites had been demonstrated in the peripheral blood. All the untreated controls died irrespective of the species of trypanosome used. Daily examinations of the blood were made in the mouse experiments for the 1st week or 10 days and at frequent intervals thereafter, while with rats and guinea pigs, the examinations usually began with 2 day intervals. The treated animals were kept under observation from 1 to 3 months.

Doses of the drug used are expressed in grams of the sodium salt per kilo of body weight. The solutions were always freshly prepared with sterile distilled water in various concentrations (0.5, 1, and 2 per cent) and the intraperitoneal route of administration was usually employed. The concentration of the drug solution in any given experiment remained a constant, the volume of the dose being the variable quantity.

Treatment of Experimental Trypanosomiasis of Mice.

For convenience of comparison, we have summarized, in the accompanying tables, the various protocols of individual experiments which were undertaken for the purpose of ascertaining the range of therapeutic action of the drug. We have included under the heading "intercurrent deaths" those animals which died from accidental

[1] Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1918, xxviii, 109.

[2] In the case of *Tr. equinum*, the infection produced in several mouse and rabbit experiments was of an unusually severe nature, a condition which may be referable to an increase in the virulence of the strain at the time of inoculation or to the strength of the inoculating dose employed.

causes within the first 15 days after treatment in the cases of *Tr. brucei*, *Tr. gambiense*, and *Tr. equinum*, and within the first 30 days in the cases of *Tr. equiperdum* and *Tr. evansi*, since it was found that relapses practically always occurred within these periods of time with our strains of these species of trypanosomes, and none of the animals listed under "intercurrent deaths" is included in the number of cured animals irrespective of the size of the dose administered.

Trypanosoma brucei.—Table I shows the results of the treatment of *Tr. brucei* infection in 75 mice with graded doses of the amide of

TABLE I.

Tr. brucei Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
1.0	2	0	0	2
0.9	2	0	0	2
0.75	2	0	0	2
0.6	3	0	0	3
0.5	1	0	0	1
0.4	2	1	0	1
0.35	1	0	0	1
0.3	11	1	0	10
0.275	5	0	0	5
0.25	19	1	2	16
0.225	13	0	3	10
0.2	13	0	8	5
0.15	1	0	1	0
Total.....	75			

N-phenylglycine-*p*-arsonic acid ranging from 0.15 to 1 gm. per kilo of body weight administered intraperitoneally. The majority of animals were treated with doses of from 0.2 to 0.3 gm., since doses above this level seemed uniformly curative, and in fact, no relapse was demonstrable with amounts of 0.275 gm. or more. Two mice, one treated with 0.4 gm. and the other with 0.3 gm., died from accidental causes within the first 15 days and consequently were not included in the number of cured animals, although in neither case could trypano-

somes be demonstrated. All the other mice which were treated with doses ranging from 0.275 to 1 gm. showed no relapse and all lived 30 days after treatment. Doses of 0.2, 0.225, and 0.25 gm. per kilo of body weight were found to exert a definite therapeutic action, although at these levels relapses occurred. With 0.25 gm., two out of nineteen mice relapsed and one died from intercurrent causes; with 0.225 gm., three relapses occurred out of thirteen mice; with 0.2 gm., there were eight relapses out of thirteen treated animals. With doses at this level, relapses usually appeared from 3 to 9 days after treatment. In some instances with doses of 0.2 and 0.225 gm., parasites were never completely cleared from the blood but they were definitely less numerous than in the blood of the untreated controls, and in addition, these mice lived longer than the controls.

TABLE II.

Tr. brucei Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intravenous Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.6	1	0	0	1
0.5	1	0	0	1
0.4	1	0	0	1
0.3	1	0	0	1
0.25	2	0	0	2
0.2	1	0	0	1
0.15	1	0	1	0
0.1	1	0	1	0
0.05	1	0	1	0
0.025	1	0	1	0
Total.....	11			

The number of mice treated intravenously and subcutaneously, shown in Tables II and III, is relatively small, yet the therapeutic results by such routes are in striking accord with those obtained by intraperitoneal administration of the drug. Of eleven mice treated intravenously with doses ranging from 0.025 to 0.6 gm. per kilo, four animals which were given doses of 0.025, 0.05, 0.1, and 0.15 gm. respectively were not cured and the blood of those receiving the three lowest doses was never cleared of parasites, while the mouse

which was treated with 0.15 gm. relapsed on the 4th day. Doses of 0.2 gm. and above were uniformly curative as contrasted with the slightly higher level of 0.275 gm. by intraperitoneal administration.

Fourteen mice were treated subcutaneously, as indicated in Table III, with doses of 0.2, 0.25, and 0.3 gm. with no relapses, although there was one intercurrent death in the first fortnight which is not

TABLE III.

Tr. brucei Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Subcutaneous Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.3	4	0	0	4
0.25	5	1	0	4
0.2	5	0	0	5
Total.....	14			

included in the number of cures. From these results, it would seem that both intravenous and subcutaneous routes of administration of the drug in *Tr. brucei* infections of mice were equally efficacious, since in both the dose of 0.2 gm. per kilo of body weight was uniformly curative, a dose which represents the lower limit of therapeutic action by intraperitoneal administration as contrasted with the curative dose of 0.275 gm. by this route.

Trypanosoma gambiense.—46 mice infected with *Tr. gambiense* were treated intraperitoneally with doses of the drug ranging from 0.125 to 0.3 gm. per kilo of body weight (Table IV). There were four demonstrable relapses; one mouse treated with 0.25 gm. relapsed on the 11th day, one with 0.15 gm. also on the 11th day, and two with 0.125 gm. on the 14th day. Of the fourteen mice which were given doses of 0.125 and 0.15 gm., three died within the first 15 days from accidental causes, three definitely relapsed, and eight animals survived the 30 day period of cure. Graded doses from 0.175 to 0.3 gm. were given to thirty-two mice, and of this number but one animal treated with 0.25 gm. relapsed. The range of therapeutic activity of the amide of *N*-phenylglycine-*p*-arsonic acid against *Tr. gambiense* infection in mice is definitely greater than against

Tr. brucei infections, since doses of 0.15 gm. failed to cure nagana mice and doses of 0.2 gm. were curative in but five out of thirteen animals.

TABLE IV.

Tr. gambiense Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.3	2	1	0	1
0.275	2	0	0	2
0.25	8	0	1	7
0.225	4	1	0	3
0.2	9	0	0	9
0.175	7	1	0	6
0.15	9	2	1	6
0.125	5	1	2	2
Total.....	46			

Trypanosoma equinum.—The results of the treatment of *Tr. equinum* infection in mice are shown in Table V. Twenty-nine mice were treated with intraperitoneal doses of 0.05 to 0.25 gm. per kilo of body weight, with no relapses at 0.2 and 0.25 gm. Doses of 0.1 and 0.15 gm. had a distinct therapeutic effect, and seven of the four-

TABLE V.

Tr. equinum Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.25	7	1*	0	6
0.2	7	3†	0	4
0.15	7	1	2	4
0.1	7	0	4	3
0.05	1	0	1	0
Total.....	29			

* Bacterial infection.

† Bacterial infection in one mouse.

teen mice treated with these doses were cured, while in some instances, the life of the uncured animal was considerably longer than that of the untreated control. A dose of 0.05 gm. had no effect whatever on the length of the infection or on the number of parasites in the peripheral blood in the single instance in which this dose was used.

Trypanosoma equiperdum.—In Table VI are given the results of the treatment of thirty-seven dourine mice with this arsenical compound in doses ranging from 0.15 to 0.5 gm. per kilo of body weight. The therapeutic action of the drug on infections in mice produced by our strain of *Tr. equiperdum* was quite irregular. Relapses

TABLE VI.

Tr. equiperdum Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.5	4	0	3	1
0.45	4	1	2	1
0.4	4	0	3	1
0.35	6	0	5	1
0.325	2	1	1	0
0.3	6	1	4	1
0.275	2	0	1	1
0.25	4	0	2	2
0.225	2	0	1	1
0.2	2	0	2	0
0.15	1	0	1	0
Total.....	37			

occurred with all doses used irrespective of size, from 4 to 15 days after treatment, and there were no cures below doses of 0.225 gm., although in every instance, even with the lowest doses, the peripheral blood was cleared of trypanosomes for several days and the mice themselves lived from 2 to 8 days longer than the untreated controls. On the other hand, there were some cures at doses of 0.2 to 0.3 gm.

Trypanosoma evansi.—Thirty mice infected with *Tr. evansi* were treated intraperitoneally with the amide of *N*-phenylglycine-*p*-arsonic acid in doses ranging from 0.15 to 0.75 gm. (Table VII).

There were definite cures at all doses above 0.15 gm. with no demonstrable relapse above 0.5 gm. Of the four mice treated with 0.75 gm., one died within the first 30 days after treatment and three were cured. With doses ranging downward from 0.5 gm., relapses occurred irregularly in from 7 to 15 days, but the trypanocidal activity of the drug was shown in all instances even with the lowest doses, since the blood was cleared of parasites for several days following treatment.

TABLE VII.

Tr. evansi Infection of Mice Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of mice.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.75	4	1	0	3
0.5	4	1	1	2
0.4	4	0	1	3
0.35	2	1	0	1
0.3	6	1	3	2
0.275	2	0	1	1
0.25	2	0	1	1
0.225	2	1	1	0
0.2	2	0	1	1
0.15	2	0	2	0
Total.....	30			

Treatment of Experimental Trypanosomiasis of Rats.

The therapeutic experiments with rats are likewise grouped in tables according to the various species of trypanosomes employed. As in the case of the mice experiments, the animals listed under "intercurrent deaths" are not included in the number of cured animals, irrespective of the time of occurrence of the death or of the size of the dose administered. Intraperitoneal administration of the drug was employed in every experiment.

Trypanosoma brucei.—Thirty-four rats infected with *Tr. brucei* were treated intraperitoneally with the amide of *N*-phenylglycine-*p*-arsonic acid in doses of from 0.2 to 1.25 gm. per kilo of body weight (Table VIII). There was but one demonstrable relapse at 0.225 gm.

in an animal which showed numerous trypanosomes in its blood 14 days after treatment. With doses of the drug as high as 0.75 gm., rats may show an early and severe intoxication from which recovery is unusual, and such was the case with the rat which was treated with 0.9 gm. and which died on the 4th day. Doses below 0.6 gm. are well borne and are uniformly curative in *Tr. brucei* infections down to 0.25 gm. However, with doses below this level, the therapeutic action of the drug is still marked as shown by the single relapse in five animals treated with 0.2 and 0.225 gm. doses.

TABLE VIII.

Tr. brucei Infection of Rats Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of rats.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
1.25	1	0	0	1
1.0	2	0	0	2
0.9	1	1	0	0
0.75	1	0	0	1
0.6	1	1	0	0
0.5	1	1	0	0
0.4	3	0	0	3
0.375	3	0	0	3
0.35	3	2	0	1
0.325	3	0	0	3
0.3	3	1*	0	2
0.275	3	2*	0	1
0.25	4	0	0	4
0.225	3	0	1	2
0.2	2	1	0	1
Total.....	34			

* Two pregnant females dying 7 and 8 days after treatment.

Trypanosoma gambiense.—Table IX shows the results of the treatment of twenty-five rats infected with *Tr. gambiense* with doses of from 0.1 to 0.3 gm. Doses of 0.2 gm. and above were uniformly curative, no relapses occurring in the fifteen animals treated with 0.2, 0.25, and 0.3 gm. per kilo of body weight. Ten animals were given 0.1 and 0.15 gm. doses, and of this number, there were three relapses and one accidental death. One rat which was treated with

0.15 gm. showed a well developed blood infection on the 18th day and died on the 25th day after treatment. The two rats which relapsed with doses of 0.1 gm. showed trypanosomes on the 10th and 18th days and died on the 25th and 36th days.

TABLE IX.

Tr. gambiense Infection of Rats Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of rats.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.3	5	0	0	5
0.25	5	2	0	3
0.2	5	2*	0	3
0.15	5	1	1	3
0.1	5	0	2	3
Total.....	25			

* Animals missing.

Trypanosoma equiperdum.—Eight rats infected with *Tr. equiperdum* were treated with the amide of *N*-phenylglycine-*p*-arsonic acid as shown in Table X. Doses from 0.25 to 0.5 gm. per kilo of body weight were used and no relapse occurred in the higher doses of 0.4 and 0.5 gm. Two rats treated with 0.3 gm. relapsed on the 8th and 16th days and one with 0.25 gm. relapsed on the 7th day. In each case, however, examination of the peripheral blood showed no parasites for several days and the animals all lived longer than the untreated controls.

TABLE X.

Tr. equiperdum Infection of Rats Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of rats.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.5	2	0	0	2
0.4	2	0	0	2
0.3	2	0	2	0
0.25	2	1	1	0
Total.....	8			

Trypanosoma evansi.—The results of the treatment of surra infection in rats are shown in Table XI. Nine rats were treated with doses ranging from 0.25 to 0.5 gm. per kilo of body weight, and no relapses were observed with doses above 0.25 gm. Of the three rats which were given 0.25 gm. of the drug, one relapsed with a heavy blood infection on the 14th day after treatment and died on the following day; a second animal which died on the 14th day showed at autopsy a large splenic tumor and was considered a probable relapse; while the third rat in this group, which died 13 days after treatment, is not counted as a cured animal, although no parasites were found in the peripheral blood and the autopsy findings were negative.

TABLE XI.

Tr. evansi Infection of Rats Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of rats.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.5	2	1*	0	1
0.4	2	0	0	2
0.3	2	0	0	2
0.25	3	1	2	0
Total.....	9			

* Pregnant female.

Treatment of Experimental Trypanosomiasis of Guinea Pigs.

A number of therapeutic experiments in guinea pigs have been done of which the example given in Table XII is typical. Doses of 0.15, 0.2, and 0.25 gm. per kilo of body weight of the compound given intraperitoneally in a 2 per cent solution were uniformly curative, no relapses having been detected during the period of observation of 3 months. The nagana infection in this particular experiment was severe, as shown by the death of the control animals on the 1st and 3rd days after treatment of the series. Other experiments in which the same and much larger doses of the drug were given showed uniformly curative results and need not be included here.

The therapeutic action of *N*-phenylglycineamide-*p*-arsonic acid upon experimental trypanosomiasis of guinea pigs is in striking accord with that obtained in mice and rats and in marked contrast to that of many trypanocidal drugs which show a greatly lessened therapeutic action in this animal species.

TABLE XII.

Tr. brucei Infection of Guinea Pigs Treated with N-Phenylglycineamide-p-Arsonic Acid 24 Hours after Inoculation. Intraperitoneal Administration.

Dose per kilo.	No. of guinea pigs.	No. of intercurrent deaths.	No. of relapses.	No. of cures.
gm.				
0.25	2	1	0	1
0.2	2	0	0	2
0.15	2	0	0	2
Total.....	6			

DISCUSSION.

As has been shown in the accompanying tables, the curative level of the amide of *N*-phenylglycine-*p*-arsonic acid is quite constant and uniform for most species of trypanosome infections in both mice and rats. Certain exceptions exist which will be referred to later, but in general, with the more virulent strains of organisms and the grade of infection which we have used, single doses of from 0.2 to 0.3 gm. per kilo of body weight given intraperitoneally 24 hours after inoculation are uniformly curative. The parasites promptly disappear from the peripheral blood within 24 hours following treatment at this level of dosage and barring death from accidental causes, the animals survive the observation period of 1 or 2 months with no return of the infection. For mice the minimum lethal dose of this drug given intraperitoneally has been placed at 2 to 2.25 gm. per kilo of body weight, and since the curative dose may be considered as 0.275 gm., we have a curative ratio of 1:8. Similarly the minimum lethal dose of the drug for rats has been estimated at 0.75 gm. per kilo of body weight, and the curative ratio for rats, therefore, is 1:3.

In addition, the range of therapeutic activity, as indicated by the

dose that will temporarily clear the blood of trypanosomes as well as effect a certain proportion of cures, varies somewhat with the species of organism. Generally speaking, with the more virulent and more rapidly fatal trypanosomes, the clearing dose closely approaches the curative dose. Thus, with *Tr. brucei* infections in mice, 0.2, 0.225, and 0.25 gm. were usually clearing doses; while with *Tr. gambiense* and *Tr. equinum*, 0.1, 0.125, and 0.15 gm. cleared the blood. On the other hand, with less virulent strains, such as *Tr. equiperdum* and *Tr. evansi*, the clearing dose is considerably below the curative level. Thus, with these strains, doses of 0.15 to 0.25 gm. cleared the blood of trypanosomes for several days, although the curative dose for both strains must be placed above 0.5 gm. The range of therapeutic activity as expressed in the size of the clearing dose is practically the same in the rat as in the mouse infections.

Different species of trypanosomes vary considerably in their resistance to therapeutic agents, and in this respect, the therapeutic action of this compound is not maintained at the constant curative level of 0.2 to 0.3 gm. per kilo of body weight. Moreover, the animal species employed may to some extent influence the control of certain infections. The curative range of 0.2 to 0.3 gm. is quite constant for *Tr. brucei*, *Tr. gambiense*, and *Tr. equinum* infections in mice and *Tr. brucei* and *Tr. gambiense* infections in rats, but with *Tr. equiperdum* and *Tr. evansi* infections in mice, the dose required to effect a cure is considerably higher. The highest dose used, 0.5 gm., failed to cure all the mice infected with *Tr. equiperdum*, although there were cures at doses as low as 0.225 and 0.25 gm. With infections of *Tr. evansi*, mice treated with 0.75 gm. did not relapse, but below this level, cures were uncertain. Both these infections, however, were more easily handled in rats than in mice, since there were no relapses with doses of 0.4 gm. in *Tr. equiperdum* and none with 0.3 gm. in *Tr. evansi* infections.

The lower level of therapeutic action of the drug is quite sharp with the more virulent species of trypanosomes. Relapses with *Tr. brucei* infections in mice occurred with 0.25 gm. (intraperitoneal administration) and increased in number with lower doses, while with *Tr. gambiense* and *Tr. equinum* infections in mice, relapses began with 0.15 gm., with the single exception of one *Tr. gambiense* mouse which

relapsed with a 0.25 gm. dose. It is possible that this relapse might have been due to a leakage of the drug at the time of treatment, since this was the only observed relapse in twenty-eight mice treated with doses of from 0.175 to 0.25 gm. The majority of relapses with these infections occurred from 5 to 12 days after treatment. The level of relapse occurrence with *Tr. brucei* and *Tr. gambiense* infections in rats is quite comparable with that observed in mice, while the time of occurrence was from 14 to 25 days after treatment. With the less virulent organisms which were used, *Tr. equiperdum* and *Tr. evansi*, the therapeutic action of the amide of *N*-phenylglycine-*p*-arsonic acid is much less clear-cut and regular. Relapses occurred in mice with doses of from 0.15 to 0.5 gm. and in general from 7 to 15 days after treatment. The therapeutic action in rats, however, is more constant and more sharply limited, for with *Tr. equiperdum* infections, there were no relapses above 0.3 gm. and with *Tr. evansi* none above 0.25 gm. The relapses with both these species in rats occurred from 8 to 16 days after treatment. It will be recalled that in all the experiments with both mice and rats, and irrespective of the species of trypanosome employed, treatment was given on the day following the inoculation of the animals and that the therapeutic results described above apply to the treatment of a 24 hour infection. The average curative level, the clearing dose, and the number and time of relapses would undoubtedly be affected with any considerable change in the time of treatment.

The therapeutic results in the treatment of experimental trypanosomiasis of guinea pigs, as shown by the low curative dose of 0.15 gm. per kilo of body weight in a severe infection, are even better than those obtained in the treatment of mice and rats and are in striking contrast to the action of certain other trypanocidal agents. The curative ratio expressed in fractions of the minimum lethal dose is 1:10, since the lethal dose for guinea pigs is 1.5 gm. per kilo of body weight given intraperitoneally or subcutaneously.

The rapidity of the trypanocidal action of the amide of *N*-phenylglycine-*p*-arsonic acid on trypanosomiasis of mice, rats, and guinea pigs is quite marked. That is, with curative doses, the drug becomes quickly available after its administration and remains in an active biological state long enough to accomplish its trypanocidal action, for

in 18 to 24 hours after the treatment of a 24 hour infection, the peripheral blood is free of parasites. Moreover, with subcurative but clearing doses, a similar speed of trypanocidal action is shown. This speed of trypanocidal activity is operative in both mice and rats, but in the case of our strains of *Tr. equiperdum* and *Tr. evansi*, the action is apparently somewhat more drastic in rats. In mice the therapeutic action extends over a wide range of doses and cures are irregularly distributed among the low as well as among the high limits of this range, while treatment of the same infections in rats reveals a more sharply limited therapeutic action. It is of interest in this connection to compare the results of the administration of the drug by different routes in the treatment of *Tr. brucei* infections of mice. By all three routes, intraperitoneal, intravenous, and subcutaneous, the sharpness of therapeutic action is maintained within the curative range and apparently the drug is as available in an active biological state by subcutaneous as by intraperitoneal or intravenous administration. The therapeutic experiments in guinea pigs are too few to admit final analysis, but apparently the rapidity and sharpness of action of the drug likewise obtain in this animal species.

One other point regarding the action of the drug should be mentioned and this is in connection with the general condition of the treated animals. Practically without exception, all the cured animals showed a marked improvement manifested by a gain in weight and by the healthy appearance of their coats which dated from the time of treatment and continued during the period of observation. The cured animals were usually in a better condition than those of the original stock. Rats which were treated with high doses approaching a lethal level and which developed toxic manifestations did not recover, but aside from this evidence of drug toxicity, the treated animals were uniformly in excellent state. The negative autopsy findings in animals that were treated with therapeutic doses of the amide of *N*-phenylglycine-*p*-arsonic acid furnish additional corroborative evidence of the non-injurious action of such doses to the host.

CONCLUSIONS.

N-Phenylglycineamide-*p*-arsonic acid is an agent of marked therapeutic action in the treatment of experimental trypanosomiasis of mice, rats, and guinea pigs. It possesses an average curative range of from 0.2 to 0.3 gm. per kilo of body weight of the sodium salt against a 24 hour infection in mice and rats produced by several species of pathogenic trypanosomes. Since the lethal dose for mice is from 2 to 2.25 gm. and for rats 0.75 gm. per kilo of body weight, we have curative ratios of 1:8 and 1:3 respectively. The curative dose for guinea pigs is 0.15 gm. per kilo of body weight, thus giving a curative ratio of 1:10. The trypanocidal activity of this compound is relatively rapid in all three animal species, for the peripheral blood is cleared of organisms within 24 hours after its administration, and in addition, the lower limits of the curative range are comparatively sharply defined. Intraperitoneal, intravenous, and subcutaneous routes of administration for all practical purposes may be considered equally efficacious in *Tr. brucei* infections of mice both as regards the speed of action of the drug and the average curative range. The administration of the drug in therapeutic amounts in all three animal species is not followed by manifestations of organic or functional injury, but, on the contrary, the general physical condition of the treated animals shows an immediate and continued marked improvement.

The therapeutic activity in trypanosomiasis of mice, rats, and guinea pigs as evidenced by the relative speed and sharpness of action, together with the curative ratio as expressed in fractions of the minimum lethal dose, and the absence of organic injury or functional disturbance following therapeutic doses are significant and characteristic features of the amide of *N*-phenylglycine-*p*-arsonic acid.

CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

Biological Series. III.

The Therapeutic Action of N-Phenylglycineamide-*p*-Arsonic Acid in Experimental Trypanosomiasis of Rabbits.

By LOUISE PEARCE, M.D., and WADE H. BROWN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 17 to 37.

(Received for publication, June 18, 1919.)

Experimental trypanosomiasis of rabbits, as has been pointed out, presents many points of similarity with naturally acquired trypanosomiasis of human beings and animals, and since the treatment offers an opportunity of obtaining fundamental information which may be of practical importance in the treatment of trypanosomal disease as it exists in nature, we have endeavored to emphasize such fundamental points in the description of the therapeutic results with the amide of *N*-phenylglycine-*p*-arsonic acid reported in this paper.

EXPERIMENTAL.

Condition of Experiments.—The majority of therapeutic experiments were carried out with strains of *Tr. brucei* and *Tr. gambiense* and a smaller number of experiments with *Tr. equiperdum*, *Tr. equinum*, and *Tr. evansi*. The infection produced by all five strains was uniformly fatal in untreated rabbits. The infecting virus in all experiments was, with one exception, obtained from stock guinea pigs in which the trypanosome strains were continuously carried, thus conforming to the principle of the influence of the animal species upon the type of infection which has been discussed in a previous paper.[1] In addition,

[1] Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1918, xxviii, 109.

in order to secure regularity of incubation as well as uniformity of character and termination of the infection in as far as this was possible, blood was taken from stock guinea pigs which showed a well developed infection and the inoculating doses of the blood suspension were given intravenously. With *Tr. brucei*, *Tr. equiperdum*, and *Tr. evansi*, 1 cc. per kilo of body weight of a + suspension was inoculated, while with *Tr. gambiense* and *Tr. equinum*, 1 cc. per kilo of a ++ to +++ suspension was used. Under these conditions of experimental procedure, rabbits infected with *Tr. brucei* showed initial signs of the disease in from 5 to 7 days and the untreated animals survived from 1 to 3 months, although the majority of controls died within the first 5 weeks, while the first signs of the infection with *Tr. gambiense* were noted 7 to 15 days after inoculation and untreated animals survived on an average of 8 weeks. The clinical course of the infection produced in rabbits by our strains of *Tr. equinum*, *Tr. evansi*, and *Tr. equiperdum* is, on the whole, comparable with that caused by *Tr. brucei*.

Rabbits thus infected were treated with the sodium salt of the amide of *N*-phenylglycine-*p*-arsonic acid at a time when the infection was well established, usually about 10 to 15 days after inoculation with *Tr. brucei* and at a somewhat longer period of time with the other strains of trypanosomes, when well marked clinical signs of the disease together with loss of appetite, weakness, and a concomitant loss of weight or even emaciation were present. In a certain number of cases, particularly in the treatment of relapses, rabbits showing a more advanced or even a chronic stage of trypanosomiasis were used.

With regard to the matter of dosage, it is important to recognize that since individual rabbits vary considerably in their reaction to trypanosomal infections as shown by the character and degree of the clinical signs, we have endeavored to utilize this factor by placing some of the animals most affected at the crucial dose levels, that is, within the curative range. The dose of the drug for each animal was calculated in grams per kilo of body weight, weighed on a balance, dissolved in sterile distilled water, and injected by a syringe into the marginal ear vein except in the few instances in which the drug was administered intramuscularly, subcutaneously, or *per os* through a stomach tube. The concentration of the drug solutions varied from 5 to 50 per cent, although in the majority of experiments a 5 per cent solution was used.

The immediate and ultimate therapeutic effect of the drug in the treatment of experimental trypanosomiasis in rabbits was closely observed throughout a period of at least 3 months and in many instances for a longer period of time, although the majority of relapses after non-curative doses of the amide of *N*-phenylglycine-*p*-arsonic acid occurred in our experiments within 2 to 5 weeks after treatment.

Determination of a Single Dose Curative Range.

Our initial experiments with the amide of *N*-phenylglycine-*p*-arsonic acid were largely concerned with the determination of the range of therapeutic action and a study of the effect of the drug within this range rather than the determination of a single curative dose or an attempt to ascertain the ideal method of treating and of curing experimental trypanosomiasis. Although the treatment of trypanosomiasis in this animal species resolves itself for the most part into the treatment of individuals, we have grouped together in Table I the results of a number of experiments which have certain features in common. In these experiments, thirty-four rabbits infected with *Tr. brucei* were treated 8 to 15 days after inoculation, at a time when all showed well marked clinical signs and symptoms of the disease. Some of the control animals died from 20 to 36 days after treatment, while others which showed extremely advanced signs at the end of 42 days were used for therapeutic purposes. The single doses of the drug employed in the treatment of these rabbits, as is shown in the table, ranged from 0.75 to 0.1 gm. per kilo of body weight and were administered intravenously in a 5 per cent solution.

There were no evidences of drug intoxication after treatment with single doses of this range and the clinical signs of trypanosomiasis were quickly influenced. Usually the acute manifestations of the infection, such as edema, capillary dilatation and congestion, and swelling of the base of the ears, eyelids, prepuce, and testicles, largely subsided within 24 to 48 hours, while the reduction of the more indurated and advanced lesions required from 2 to 4 days. The immediate clinical results of the treatment are illustrated in Figs. 1 to 7. Apart from the outward signs of the disease, the animal's general condition improved markedly following the injection of the drug as shown by

a rapid return of appetite and an increase in weight, so that within a few days emaciation had given place to a more or less well nourished condition, while in the succeeding weeks, a normal increase in weight occurred.

The final results in this series are given in Table I. There were no relapses among the twelve rabbits treated with doses of both 0.35 and 0.25 gm. per kilo of body weight, while with the intermediate dose of

TABLE I.

Results Obtained in the Treatment of Rabbits Infected with Tr. brucei with Single Doses of N-Phenylglycineamide-p-Arsonic Acid, Administered Intravenously.

Dose per kilo.	No. of rabbits.	No. of intercurrent deaths.	No. of relapses.	No. of cures.	Percentage of cures.
gm.					
0.75	1	0	0	1	100
0.4	2	0	0	2	100
0.35	6	2*	0	6	100
0.3	7	0	1	6	86
0.25	6	3†	0	6	100
0.2	10	1‡	3	7	70
0.1	2	0	2	0	0
Total.....	34				

* One was killed 71 days after treatment; extensive ringworm. One died 73 days after treatment; middle ear abscess.

† One died the day before the series was discarded; hemorrhagic septicemia. One died 71 days after treatment; acute pneumonia. One was killed 71 days after treatment; extensive ringworm.

‡ Killed 77 days after treatment; bacterial infection.

0.3 gm. per kilo, there was one relapse out of seven animals, or 86 per cent of cures. Furthermore, with a lower dose of 0.2 gm. seven out of ten rabbits were cured (70 per cent), and finally, the two rabbits treated with the very low dose of 0.1 gm. relapsed, although the marked therapeutic activity of the drug at this level is shown by the fact that with rabbits so treated, regression and healing of the lesions took place very rapidly and the relapses did not occur until 11 and 21 days after treatment. There were six incidental deaths among the thirty-four rabbits included in this table, but since they occurred over 10 weeks after treatment and were in no way associated with evidences

of trypanosomiasis, we have not excluded them from the number of cured animals.

The outstanding feature of the treatment of an acute, progressive, and well established *Tr. brucei* infection in rabbits with single doses of the amide of *N*-phenylglycine-*p*-arsonic acid as shown in Table I is a well marked sharply limited curative range of therapeutic action with doses varying from 0.2 to 0.35 gm. per kilo, and that within these limits, the percentage of cures is, on the average, high. Thus, there were twenty-nine rabbits treated with doses of 0.2, 0.25, 0.3, and 0.35 gm. per kilo of body weight and of these four relapsed and twenty-five, or 86.2 per cent, were cured. In addition, both upper and lower limits of action of the curative range are sharply marked—with doses of 0.2 gm., there were 70 per cent of cures and with the lower dose of 0.1 gm., there were no cures although this amount of the drug possessed a marked therapeutic effect, while, on the other hand, doses of 0.35 gm. or higher were uniformly curative. If we consider that the minimum lethal dose of the drug for rabbits is from 0.75 to 0.9 gm. per kilo of body weight when given intravenously and that the curative range of single doses is from 0.25 to 0.35 gm., we have a curative ratio of from one-third to one-half as expressed in fractions of the minimum lethal dose.

Much the same range of curative action is operative in rabbit trypanosomiasis produced by *Tr. gambiense* as is shown in Table II. In this series, eighteen rabbits were treated intravenously with doses of the amide of *N*-phenylglycine-*p*-arsonic acid of 0.35 to 0.15 gm. per kilo of body weight. There was a very noticeable regression of the signs of the infection on the 1st and 2nd days after treatment, and no detectable signs remaining on the 5th day, as may be seen in Figs. 8 and 9. Two out of the three rabbits treated with 0.15 gm. per kilo relapsed 20 and 33 days after treatment, and there was one relapse at 0.25 gm. 26 days after treatment, but the eleven rabbits treated with the other three doses of 0.2, 0.3, and 0.35 gm. per kilo were cured. Thus the curative range of the compound in *Tr. gambiense* infections of rabbits may be considered to be from 0.2 to 0.3 gm. per kilo when administered as single doses intravenously—or from one-fourth to one-third of the minimal lethal dose.

Our experience with the effect of this compound upon rabbit trypanosomiasis produced by other species of trypanosomes is limited

so that we are unable to draw final conclusions as to the ultimate curative range of the drug in these particular infections. At the time the experiments were done, the infection produced in these animals by our strain of *Tr. equinum* was unusually severe as was also the case in certain mouse experiments, and in addition, the upper range of the doses used was apparently too low. However, single doses as small as 0.2 gm. per kilo of body weight caused a complete disappearance of the acute signs of the disease in 3 days, although relapses eventually occurred with the doses 0.15, 0.2, 0.25, and 0.3 gm. per kilo. It was obvious that larger doses should have been used or a different system of treatment followed to effect a permanent cure in an infection as

TABLE II.

Results Obtained in the Treatment of Rabbits Infected with Tr. gambiense with Single Doses of N-Phenylglycineamide-p-Arsonic Acid, Administered Intravenously.

Dose per kilo.	No. of rabbits.	No. of intercurrent deaths.	No. of relapses.	No. of cures.	Percentage of cures.
gm.					
0.35	3	0	0	3	100
0.3	4	0	0	4	100
0.25	3	1*	1	1	50
0.2	5	1†	0	4	100
0.15	3	2‡	2‡	1	33⅓
Total.....	18				

* Died 6 days after treatment; bacterial infection.

† Died 20 days after treatment; gastroenteritis.

‡ Relapse plus bacterial infection 20 and 33 days after treatment.

severe as was the case in this particular experiment, since the control rabbit died the day after the animals in the series were treated and relapses occurred with such promptness and severity.

Two rabbits heavily infected with *Tr. equiperdum* which were treated with single doses of 0.5 and 0.3 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo of body weight, 36 days after inoculation, at a time when the disease was advanced, showed a prompt recovery from the clinical signs as is illustrated by Figs. 12 and 13. Both rabbits subsequently relapsed on the 47th and 57th days after treatment but were successfully retreated with repeated doses of the drug with no ultimate recurrence of the infection.

The therapeutic effect of the compound upon *Tr. evansi* in rabbits was studied in but one animal. The infection was well established on the day of treatment, 36 days after inoculation, as is shown in Fig. 14, when 0.3 gm. per kilo was administered intravenously. There was very little change in the clinical signs during the first 48 hours but on the following days, the improvement was marked (Figs. 15 and 16). Thick crusts about the nose and upper lips had shelled off in 10 days time and by the end of the 3rd week, the skin in these areas was healthy in appearance and the hair had begun to return (Fig. 17). There was no relapse and the rabbit was in excellent condition when it was discarded 113 days after treatment.

Summing up the results of the treatment of trypanosomiasis with single doses of the drug, we may say that with infections of average severity and duration produced by *Tr. brucei* and *Tr. gambiense*, the drug exercises a prompt and lasting therapeutic action in doses of from 0.2 to 0.35 gm. per kilo of body weight, and, furthermore, a marked therapeutic effect of some duration in doses of 0.1 and 0.15 gm. per kilo of body weight. Although the experimental evidence with regard to *Tr. evansi* infection in rabbits is insufficient for final conclusions, it is probable that the same range of curative action would apply to such rabbit infections as are produced by the particular strain of surra with which we have worked. On the other hand, single doses of the compound which fall within this curative range failed to cure *Tr. equinum* and *Tr. equiperdum* infections in rabbits. However, it should be pointed out that in both these experiments, especially with *Tr. equinum*, the disease was extremely marked and more advanced at the time of treatment than was the case with the other three infections, and it is possible that with more comparable experimental conditions, a certain proportion of cures could be obtained with single doses falling within the curative range of 0.2 to 0.35 gm. per kilo of body weight.

In addition to the above experiments, a series of twelve rabbits with a very severe *Tr. brucei* infection of 23 days duration was treated intravenously with large single doses in order to ascertain the reaction of extremely ill animals to the drug as compared with that of normal animals. The dose of 0.9 gm. per kilo of body weight caused toxic symptoms in the three rabbits so treated, with death on the 2nd and

3rd days, while a similar result followed the dose of 0.75 gm. in two out of three rabbits; with 0.6 gm., only one rabbit of the three became toxic and eventually recovered, and the dose of 0.5 gm. caused no toxic disturbances whatever. From the above results, it is evident that the tolerance of very ill rabbits suffering from a severe acute trypanosomiasis infection compares very favorably with that of normal animals to single large doses of *N*-phenylglycineamide-*p*-arsonic acid administered intravenously.

Treatment of Initial Infections with Repeated Doses.

In many respects, the treatment of rabbit trypanosomiasis with repeated small doses of the amide of *N*-phenylglycine-*p*-arsonic acid more nearly meets conditions as they are found in nature, and in addition the control and ultimate cure of severe and long standing infections which might not yield to a single dose treatment may often be successfully accomplished by this system of therapeutic procedure. This is due in part to the fact that with the debilitated and weak condition of the animal host, it may not be wise to give a single dose of sufficient size and probably in part also to the chronic and indurated nature of the lesions present, the penetration of which may be difficult and may require considerable time. By observing the action of single small doses of the drug, we form an idea of their effect and duration. Obviously, the repetition of a dose should occur before the effect of the previous dose has ceased, and we consider the proper spacing of doses as important a factor in this connection as the number and size of the doses. Finally, all these factors must be largely determined by the physical state of the diseased animal as well as by the age, severity, and type of the infection.

We have treated a number of rabbits infected with *Tr. brucei* with three doses of the compound ranging from 0.2 to 0.05 gm. per kilo repeated at various intervals of time, of which the following are typical examples. Two rabbits infected with *Tr. brucei* were treated 11 days after inoculation, with three doses of 0.2 gm. per kilo repeated at 2 day intervals. The disease was well established with marked typical clinical signs at the time of treatment. Both animals recovered promptly with no signs of drug intoxication and no return of the

infection. Two other rabbits in the same series treated with three doses of 0.15 gm. at 2 day intervals likewise showed a prompt and complete disappearance of the clinical signs of the infection but one animal eventually relapsed 30 days later. Another animal which was given three doses of 0.1 gm. at 2 day intervals remained free from any signs of the infection for 186 days at which time it was discarded as cured. The last rabbit in this series which was given three doses of 0.05 gm. relapsed 13 days after treatment had been begun.

A modification of the spacing of repeated small doses was followed in the case of another animal which was treated 14 days after inoculation with *Tr. brucei*; the initial dose was 0.1 gm. per kilo. 8 days after treatment, the return of clinical signs of trypanosomiasis was observed about the head and nose, and the dose of 0.1 gm. per kilo was repeated 2 days later. The clinical signs cleared up promptly within 4 days and the animal remained in good condition without relapse during the period of observation following treatment.

In the experiments just cited, the infections were of an acute, rapidly progressive type. The effect of treatment with repeated doses of this arsenical upon a slowly developing severe infection of long standing is illustrated in Figs. 18 to 23. In this instance, the rabbit was inoculated with *Tr. brucei* in the usual manner but the infection was relatively slow in developing and treatment was not begun until 48 days after inoculation, when three doses of 0.2 gm. were given intravenously on succeeding days. At this time, the animal was extremely weak and emaciated, the ears, eyelids, face, nose, and external genitalia were swollen and indurated, and in addition, there were a mucopurulent exudate and scab formation about the eyes and nose (Fig. 18). An immediate improvement in the clinical manifestations of the disease was seen after treatment, although there was a loss of weight for the first 5 days, but by the 7th day this had largely been regained. Within the week following treatment, the scabs about the ears and around the eyes and end of the nose and lips had scaled off almost entirely, leaving a fairly healthy looking skin, the indurated swellings about the head and genitalia had greatly subsided, and the general condition of the rabbit had improved remarkably as is shown in Figs. 19 and 20. By the end of 3 weeks, the change in the appearance of the rabbit as contrasted with its condition on the day of treatment was

quite striking (Fig. 22), and in 6 weeks, a small bald patch at the base of the left ear was the only residual sign of a previous lesion (Fig. 23). The recovery from a severe and long standing infection under the system of three small doses of 0.2 gm. per kilo repeated at daily intervals was complete and lasting, for there was no subsequent relapse during the period of observation of 195 days.

The treatment of initial infection with *Tr. brucei* in rabbits, therefore, may be successfully accomplished by a system of repeated small doses provided that the repetitions occur well within the time of duration of action of the previous dose. The value of this system of therapy is especially marked in instances of greatly weakened animals or in those in which chronic indurated lesions are pronounced.

Treatment of Relapses with Single Doses.

The successful treatment of trypanosomiasis relapses in rabbits, many of which were intentionally produced as test objects, is based largely upon the severity and duration of the relapse and the general physical condition of the rabbit. While the factor of fastness of trypanosomes due to previous treatment unquestionably plays a part in the blood infections of mice and rats and must be considered to a certain extent in the tissue infection of rabbits, it is our opinion that the severity and duration of the relapse as manifested by the signs and symptoms of the actual infection, together with the general physical state of the rabbit host, play a more important rôle. It is obvious that the treatment of a relapse is almost wholly the treatment of individuals, and hence no rules of procedure can be laid down; but because of the inherent condition imposed by the infection, we believe that in most cases relapses can best be handled by a system of repeated doses, the size and time of repetition of which are necessarily determined by the extent, severity, and age of the disease together with the physical condition of the animal host. However, if a single large dose is used, it must of necessity cause no serious degree of intoxication or pathological injury, and yet at the same time, it must be of sufficient strength to penetrate diseased tissues of more or less induration and in like manner to act as a trypanocidal agent for a sufficient length of time. A number of relapses in rabbits have

been treated with single doses of this arsenical, of which the following will serve as examples.

The appearance of a *Tr. brucei* relapse of a week's duration at the time of retreatment is shown in Fig. 24. This rabbit was originally treated intravenously with three doses of 0.05 gm. per kilo of the drug which had served to clear up all outward manifestations of the disease, but 13 days later, definite signs of a relapse were noted. The infection was allowed to progress for a week at which time the clinical signs had rapidly increased both in degree and extent. The general physical condition of the rabbit was good. The entire left side of the face was greatly swollen and the left eye completely closed; the upper eyelid was very puffy, and protruding and escaping from between the margins of both lids, which were reddened with considerable loss of hair, was a slightly purulent exudate. The right upper eyelid, right cheek, and upper lip were also slightly swollen. Both ears, especially the left, which drooped markedly, were swollen, inflamed, and edematous and the right testicle was somewhat indurated. The dose selected for retreatment in this instance was 0.5 gm. per kilo of the amide of *N*-phenylglycine-*p*-arsonic acid given intravenously; it was followed by no toxic symptoms whatever. There was a most marked reduction of the clinical signs on the 2nd day; in a week's time the appearance of the rabbit was quite normal as is shown in Fig. 25, and this continued with no return of the infection during the period of observation of 166 days.

Another nagana rabbit which had been treated intravenously with the single dose of 0.29 gm. of the drug per kilo of body weight relapsed 26 days after treatment and the signs of the disease increased extremely rapidly for the succeeding 8 days. A single dose of 0.4 gm. per kilo of the drug was given intravenously. This was followed by a marked immediate improvement in the clinical signs of the infection and in 10 days the animal had regained its original weight and its appearance was entirely normal. There was no further relapse during the 149 day period of observation.

In addition to the treatment of rabbits infected with *Tr. brucei* which had relapsed after the initial treatment with the amide of *N*-phenylglycine-*p*-arsonic acid, a number of animals were treated that had relapsed from initial treatment with single doses of arsphenamine

and neoarsphenamine (German salvarsan and neosalvarsan), ranging from 0.005 to 0.02 gm. per kilo of body weight. For purposes of comparison, the original series which included these rabbits was conducted under the same conditions as were the parallel experiments in which the amide of *N*-phenylglycine-*p*-arsonic acid was used, and the infection was of the same acute nature with marked clinical signs at the time of treatment. The animals which relapsed after the treatment with arsphenamine and neoarsphenamine were held until the infection had progressed to an extreme degree with conspicuous clinical signs and a generally poor physical state of the animals; they were then treated with single doses of *N*-phenylglycineamide-*p*-arsonic acid. Five animals were given 0.5 and eight were given 0.4 gm. per kilo of body weight and all promptly recovered from the signs of the infection, with no evidences of drug intoxication and with a very noticeable and rapid improvement in their physical condition. Three rabbits in this group were killed 34, 47, and 56 days after treatment because of extensive ringworm or middle ear disease, but at autopsy no evidence of trypanosomiasis was found and none of the remaining ten rabbits suffered a relapse from the retreatment with *N*-phenylglycineamide-*p*-arsonic acid.

From the experimental evidence, therefore, it is seen that rabbits infected with our strain of *Tr. brucei* which have relapsed with severe clinical signs after insufficient treatment with the amide of *N*-phenylglycine-*p*-arsonic acid, arsphenamine, and neoarsphenamine may be permanently cured with single doses of 0.4 and 0.5 gm. per kilo of body weight of the amide of *N*-phenylglycine-*p*-arsonic acid.

Treatment of Relapses with Repeated Doses.

In the treatment of relapses with repeated doses, the chief factors of importance to be considered are first, the severity and type of the infection and the general physical condition of the rabbit. If the relapse is long standing with chronic indurated lesions, a single dose of the drug may not penetrate the lesions sufficiently or operate over the necessary length of time required for resolution of such lesions. Moreover, as is frequently the case with this type of chronic relapse, the physical state of the rabbit may be very poor and a single large dose

of the drug might under these conditions prove somewhat dangerous. Hence, from a consideration of these factors, it would seem that a system of small repeated doses, properly spaced, is the most rational system of treating chronic trypanosomal relapses of rabbits. The size of the initial dose or doses should be large enough to exert some influence upon the infection and its lesions, and subsequent doses should be given before the effect of the previous dose has completely worn off. Consequently a knowledge of the general effect and duration of action of various sized doses of this arsenical, as well as accurate and frequent clinical observations of the rabbits under treatment, is essential to a satisfactory outcome with this system of therapy. In this connection, it is important to bear in mind that rabbits as well as other animal species exhibit a high degree of tolerance for the drug as has been shown elsewhere,[2] and this attribute which exists in rabbits infected with trypanosomiasis as well as in non-infected animals may be used to great advantage in the treatment of trypanosomiasis, both of the initial infections and of the relapses. Our experience in handling relapses with repeated doses of this drug has been extremely varied because we have purposely endeavored to ascertain what might be accomplished with various sized doses given at various intervals of time under the many different conditions imposed by the infection rather than an attempt to effect a cure in every case. We have selected certain typical protocols which will illustrate some of the essential points in question.

Fig. 26 shows the appearance of an 8 day relapse in a rabbit which had been treated 34 days before with 0.02 gm. of arsphenamine (German salvarsan) per kilo of body weight. The signs of the relapse increased rapidly and at the time of retreatment with the amide of *N*-phenylglycine-*p*-arsonic acid, the rabbit was not in a particularly good general condition. The face was markedly swollen and indurated, both eyes were closed, the base of both ears was involved in the inflammatory process, both testicles were swollen and indurated, and the prepuce was extremely swollen and congested. Three doses of 0.15 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid were given intravenously on successive days. There were no evidences of drug intoxication whatever, the general condition improved immediately, and

[2] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1919, xxx, 417.

the clearing up of the clinical signs progressed rapidly as is seen in Fig. 27, which shows the appearance 6 days after treatment had been begun. At this time there remained only a slight swelling and induration of the face and a slight degree of resistance of normal size testicles. These residual signs had completely disappeared on the following day. Unfortunately no opinion of the ultimate therapeutic success of the treatment was possible in this particular case as the animal succumbed to a bacterial infection 24 days after treatment.

Fig. 28 illustrates the appearance of a relapse of 15 days duration in a rabbit that had been treated 49 days previously with 0.02 gm. of neoarsphenamine (German neosalvarsan) per kilo of body weight. There was a very marked swelling of the face and the base of the ears, particularly of the left, an indurated swelling of the eyelids with crust formation, a purulent exudate, loss of hair and complete closure of both eyes, a mucoserous nasal discharge, and a moderate involvement of the genitalia. In addition, the animal was very thin and weak. A dose of 0.5 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo was given intravenously and was followed by a marked general improvement and gradual disappearance of the signs of the infection with an accompanying gain in weight, although the rabbit still remained weak and middle ear disease developed. 2 weeks after the first dose of the drug had been given, a second dose of 0.5 gm. per kilo was given intravenously with no evidences of drug intoxication but with a decided improvement in the animal's general condition, although the local condition in the right middle ear continued to progress, causing a marked twisting of the head to the right. In the course of recovery from the trypanosomal infection, a large portion of diseased skin over the bridge of the nose and about the eyes gradually desquamated, leaving a healthy looking but bare surface denuded of hair. The growth of new hair in these areas was quite remarkable as is shown in Fig. 29. There was no later evidence of trypanosomiasis observed in this rabbit either clinically or at autopsy, and despite the middle ear abscess which was eventually the cause of death 59 days after the treatment of the relapse, the general physical condition remained excellent.

We have selected two examples of the treatment of nagana relapses of a more prolonged and chronic type which are generally considered to be much more difficult of permanent cure than the types previously

considered in this section. The first of these rabbits was originally treated intravenously with 0.2 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo of body weight which sufficed to clear up all signs of the infection. However, 27 days later, the diagnosis of a probable relapse was made because of signs about the eyelids, and although at the end of a week's time, the clinical signs of trypanosomiasis were outspoken, the relapse was allowed to progress and the rabbit was not treated until 22 days after the first signs had been noted. The appearance of the head of the rabbit on the day of retreatment is shown in Fig. 30. There was a marked edematous swelling of the face involving the cheeks and eyelids and the eyes were almost closed; both upper lips were extremely swollen and congested with some loss of hair and scab formation; the lower portions of the ears were considerably indurated and very hot to the touch and at the base of the right ear was a necrotic area the size of a silver half dollar. The prepuce was edematous, congested, and somewhat swollen and both testicles although not enlarged were indurated. The general physical condition of the rabbit was fairly good. Two doses of 0.4 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid given a week apart caused a marked improvement in the rabbit's condition as shown in Figs. 31 and 32, but 3 days after the second dose, there was a swelling of the left cheek and both left eyelids, with a slight mucopurulent discharge from the eye. The succeeding four doses (0.6, 0.5, 0.5, and 0.75 gm. per kilo) given over a period of 28 days were eventually effective in clearing up the clinical signs (Figs. 33 to 35), but a relapse ultimately occurred during the summer. Since the total amount of the drug administered was in our experience sufficiently large to cure a relapse of this duration and type, it would seem that the failure was due to the incorrect spacing of the doses which were too far apart. It is probable that if the first three doses had been given on successive or even on alternate days, a permanent cure would have been obtained.

As a contrast to the system of treatment of a relapse pursued in the preceding experiment which failed to effect a permanent cure, the following instance is given of the successful treatment of a chronic and very advanced relapse, a type of infection generally considered extremely difficult of cure. A rabbit infected with *Tr. brucei*, which had been treated in the spring, relapsed during the summer, and at the time of retreatment, about 3 months later, presented an extreme

picture of a long standing chronic relapse (Figs. 36 and 37). A large portion of the face including the area around both eyes and the bridge of the nose was practically devoid of hair. The skin had lost its elasticity, was quite firm, resistant, and indurated, and there were numerous small scabs over the surface, especially around the right eye. The base of the right ear was also swollen and indurated and there was a small raw thickened area on the right hind foot covered with reddish yellow scabs. The prepuce was markedly swollen and quite firm; both testicles although not enlarged were very firm and indurated. The rabbit was weak but in a fairly good state of nutrition. Treatment was begun with the injection of 0.75 gm. of the drug per kilo of body weight and repeated on the 4th and 6th days—a dose which we believed was unnecessarily large, yet the reaction and effect of which we wished to determine in this particular type of relapse.

The therapeutic effect of the drug upon the clinical lesions was most marked as is shown in Figs. 38 to 40. There was no loss of weight, the rabbit's general condition was very noticeably improved, and, moreover, there was no indication of any drug intoxication at any time. 1 week after treatment had been begun, most of the scabs about the base of the right ear, around both eyes, and over the face had scaled off, leaving a smooth, soft, elastic skin denuded of hair but otherwise quite healthy looking, and the involved area on the right hind foot had a similar appearance. The prepuce was practically normal and the testicles were rapidly becoming so.

The further changes in this rabbit during the succeeding 10 days were quite remarkable as are shown in Figs. 41 and 42. The eyelids, nose, face, and base of the right ear were no longer swollen or indurated and the rapid growth of hair in the bare areas was quite conspicuous. The affected area on the right hind foot was somewhat pinkish with a few yellowish pink scabs but with a beginning growth of fine hair. The external genitalia appeared normal, although on palpation the scrotum on both sides was still somewhat diffusely thickened and the testicles were atrophic. On the 11th and 14th days, two additional doses of 0.75 gm. per kilo of the drug were given intravenously for the purpose of determining any degree of acquired tolerance or any signs or symptoms of drug intoxication that these

additional large doses might cause, although from the point of view of the infection, we were confident that they were unnecessary. Apparently, however, they had no untoward effect whatever and the rabbit continued to improve very noticeably. By the end of the 3rd week, there were no residual lesions indicative of the trypanosomiasis infection except one or two small patchy areas below the inner canthus of each eye and just above the tip of the nose where the hair was thinner than elsewhere (Fig. 43), but in a short time, these areas had resumed a normal appearance. This particular rabbit has been held in the laboratory 3 years and there has been no return of the infection.

The treatment of trypanosomal relapses in rabbits with this compound depends largely upon two important factors, the general physical state of the rabbit and the extent and severity of the infection, and these conditions can in our opinion be most adequately handled by a system of repeated doses. Since the factor of tolerance for the amide of *N*-phenylglycine-*p*-arsonic acid is exhibited to a considerable degree in rabbits, the size of succeeding doses may be greatly increased if necessary and the number of dose repetitions may also be large. However, it is of essential importance that the succeeding doses should be repeated well before the effect of the previous dose has worn off.

Administration of the Drug by Routes Other than the Intravenous.

The great majority of experiments with the amide of *N*-phenylglycine-*p*-arsonic acid in the treatment of rabbit trypanosomiasis, including those described above, were carried out by the intravenous administration of the drug, since this route offers uniform and constant conditions from the point of view of actual administration as well as development of drug action. Under such conditions, a comparison of the effect of different sized doses and a final appraisal of the action of the drug from the point of view of a permanent cure may be more accurately made. However, since it may be desirable to employ routes of administration other than the intravenous, from the standpoint of actual usage and ease of manipulation, a few rabbits infected with *Tr. brucei*, of which the following are examples, were treated subcutaneously, intramuscularly, and *per os*, in order to ascertain the general effect and duration of certain selected doses upon initial infections and relapses.

Two rabbits with a 14 day nagana infection and showing well marked clinical signs of the disease were treated with 0.1 gm. of the drug per kilo of body weight subcutaneously. The acute inflammation and edema about the head and external genitalia were materially reduced and only slight signs remained 7 days after treatment, but on the 8th day, they became increased in the first rabbit. Consequently both animals were treated on the 8th and 10th days with 0.1 gm. per kilo, again subcutaneously. The clinical signs regressed completely in the first animal during the following week and were absent for 3 weeks, at which time they recurred with considerable severity; in the case of the second rabbit, in which the infection was still in abeyance at the time of the second and third doses, the clinical signs were absent for 6 days after the second treatment; they then recurred and steadily increased in severity and extent. Two other rabbits in this group were treated subcutaneously with a dose of 0.2 gm. of the drug per kilo of body weight which was not repeated. One rabbit was permanently cured and the other relapsed on the 36th day. A fifth animal with a long standing infection of 48 days duration showing marked chronic lesions about the head and external genitalia with considerable loss of weight was treated with 0.5 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo subcutaneously. There were no signs of drug intoxication and no inflammation or induration about the site of injection; the regression of lesions proceeded somewhat more slowly than after intravenous administration of the drug, but at the end of 2 weeks they had subsided and the animal appeared normal except for a few bald patches about the head. No further signs of trypanosomiasis were observed for 41 days, at which time the outer margin of the right ear showed signs of early involvement and 2 days later a second dose of 0.25 gm. per kilo, one-half the original dose, was given subcutaneously for the purpose of ascertaining the effect upon the local lesion and upon the general course of the infection of a single dose much smaller than the original one. There was a very noticeable improvement of the ear lesion on the following day. A portion of the scab scaled off and the induration of the entire margin was distinctly decreased; on the 5th day after treatment, the ear appeared normal. There were no further indications of a relapse but unfortunately the rabbit developed an acute, extensive ringworm and

had to be killed 35 days after the second dose so that the question of a permanent cure is problematical. The rapid healing of the local ear lesion, however, and the fact that the relapse was treated soon after its appearance, although with a comparatively small dose, and that as long a time as 35 days elapsed without signs of a relapse either clinically or at autopsy are all factors indicative of a probable cure.

Seven rabbits were treated by the intramuscular route of administration. Two animals which were treated 14 days after inoculation with *Tr. brucei* received single doses of 0.2 gm. of the drug per kilo. The speed of action of the drug upon the well marked characteristic clinical lesions of the infection as seen in these two animals was apparently quite comparable with that observed after intravenous administration, and a permanent cure was effected in both rabbits. Two other rabbits of the same series were treated with an initial dose of 0.1 gm. per kilo which was repeated on the 8th and 10th days. One animal was cured but the other showed marked lesions of the disease 6 days after the last dose and was treated again with three doses of 0.1 gm. per kilo of body weight given intramuscularly on successive days with a marked, though temporary effect. In this instance, it is probable that the infection was originally not cleared by the first three doses and consequently either the second course of treatment was not given soon enough to effect a permanent cure or the amount of drug was not large enough. Another rabbit was treated 42 days after infection with *Tr. brucei* with three doses of 0.1 gm. per kilo of body weight intramuscularly on successive days. At the time of treatment, the lesions about the ears, eyes, face, nose, and lips were extremely marked and the animal itself was in only a fair physical condition. Regression and healing of the lesions about the head took place very satisfactorily and there were no signs of drug intoxication, but a subcutaneous abscess developed at the site of injection in the lumbar region and gradually increased in extent. The rabbit was killed 25 days after treatment. There were no clinical signs of a relapse at this time and the autopsy findings were negative. Finally, two rabbits with severe relapses after previous treatment—one with 0.015 gm. per kilo of neoarsphenamine given intravenously and the other with three doses of 0.1 gm. per kilo of the amide of *N*-phenylglycine-*p*-arsonic acid given subcutaneously—were treated intramuscularly with

larger doses of the latter drug. The first, which received 0.5 gm. per kilo of body weight, showed a rapid regression and healing of the lesions of the infection and was permanently cured; the other rabbit was given an initial dose of 0.3 gm. per kilo of body weight and 3 days later 0.5 gm. per kilo with no sign of drug intoxication and a very marked improvement in the signs of the infection. The ultimate result of the intramuscular administration of the drug cannot be determined in this case, for the succeeding treatment 11 days later was by mistake given intravenously.

Finally, an example of the therapeutic administration of the amide of *N*-phenylglycine-*p*-arsonic acid by mouth may be given for completeness, although the number of trypanosomiasis rabbits treated by this route is too small to admit of any conclusion regarding the effect and final result of various sized doses. A severe initial infection of 8 days duration with well marked clinical signs was treated with a single dose of 0.75 gm. per kilo of body weight given *per os* immediately after a small dose of sodium bicarbonate. There were no untoward effects following the administration of the drug; the clinical signs of the infection regressed and healed in the usual time and the rabbit was permanently cured.

While the number of rabbits treated with the amide of *N*-phenylglycine-*p*-arsonic acid administered by the subcutaneous, intramuscular, and *per os* routes is too few to admit of final conclusions as to their comparative value and usefulness, they demonstrate that even relatively small doses of the drug are highly active therapeutically when given intramuscularly and to a somewhat less extent subcutaneously, while in one instance a large dose given by mouth cured a severely infected animal.

SUMMARY.

In the treatment of experimental trypanosomiasis of rabbits with subsequent appraisal of the value of the therapeutic agent used, there are certain experimental factors including uniform infecting strains of trypanosomes and the observation of general procedures of method and time of inoculation conditioned by the infection itself which must be taken into account. The conspicuous and characteristic clinical

signs and symptoms seen in rabbit trypanosomiasis serve as criteria of the severity and duration of the disease, and it is obvious that the infection should be well established before treatment is instituted. For the same reason, before the question of a permanent cure can be established, treated rabbits should be kept under observation for a sufficient period of time, which with the species of organisms that we have used is at least 3 months.

The therapeutic results with the amide of *N*-phenylglycine-*p*-arsonic acid were obtained in rabbits which showed well marked clinical signs of a definitely established disease, and in many instances the infection was extremely advanced and of prolonged duration. The five species which we have employed, *Tr. brucei*, *Tr. gambiense*, *Tr. equinum*, *Tr. equiperdum*, and *Tr. evansi*, are uniformly fatal in rabbits. With the usual acute, actively progressing infection of from 1 to 2 weeks duration produced by our strain of *Tr. brucei*, the drug has a curative range of from 0.2 to 0.35 gm. per kilo of body weight, when administered intravenously in single doses, or from one-third to one-half the minimal lethal dose. Of the twenty-nine rabbits treated with doses falling within this range, twenty-five, or 86 per cent, were permanently cured and there were no relapses observed with doses above 0.3 gm. The infection produced by our strain of *Tr. gambiense* is controlled by a slightly lower dose, since there were no relapses with single doses of 0.3 gm. and a single dose of 0.15 gm. effected a cure in one of three rabbits so treated. The therapeutic experiments with *Tr. equinum*, *Tr. equiperdum*, and *Tr. evansi* are too few to admit of final conclusions, but apparently from the evidence at hand, much the same curative range is operative in *Tr. evansi* infections, while larger doses or a different system of treatment should have been employed in the treatment of rabbits infected with our strains of *Tr. equinum* and *Tr. equiperdum*.

In addition to the ultimate curative results obtained with single doses within the curative range, it is important to consider the marked therapeutic action with smaller single doses, as shown by the rapid regression and healing of the clinical lesions of the acute infections produced by all five species of trypanosomes together with a marked improvement in the general physical state of the animal. Moreover, large single doses, above those of the so called curative range, caused no disturbance of a toxic nature and were apparently well borne.

A system of repeated dose therapy may be employed with advantage in the treatment of both initial and relapsed infections in rabbits, especially in those instances in which there is induration or even necrosis of tissues with weakness and emaciation of the animal host. The factor of time of repetition or the spacing of doses is in our experience as important as that of size of the dose employed and depends upon the rate, degree, and duration of action of the particular dose of the drug in question. Since the amide of *N*-phenylglycine-*p*-arsonic acid apparently possesses the power of tissue penetration to a marked degree, it is desirable to give the second dose within a short time after the first in order that it may have a full opportunity for the immediate and complete development of its action. The repetition of small doses such as 0.15 gm. per kilo of body weight on successive or alternate days has given successful results as regards both the immediate regression and healing of lesions and ultimate permanent cures in severe, chronic infections. It is possible, however, to administer increasingly large doses, if this is necessary, since infected as well as normal rabbits exhibit a remarkable tolerance to repeated large doses of the drug. The therapeutic activity of small doses administered intramuscularly is quite comparable with that observed after similar doses given intravenously, as indicated by the rate of regression and healing of clinical lesions, while such effects proceed somewhat more slowly after subcutaneous injections. Permanent cures have been obtained in *Tr. brucei* infection with intramuscular and subcutaneous administration of single doses of from 0.2 to 0.5 gm. of the drug per kilo of body weight and in other instances with three repeated doses of 0.1 gm. per kilo given intramuscularly. One severely infected rabbit which received 0.75 gm. per kilo *per os* immediately following a small dose of sodium bicarbonate was also cured.

The therapeutic experiments here reported represent only a portion of those carried out with *N*-phenylglycineamide-*p*-arsonic acid and the scope of the present paper does not permit a detailed description of the many phases of the experiments or a full discussion of the various factors involved and the results obtained, all of which we hope to publish at some future time.

EXPLANATION OF PLATES.

The figures are reproductions of untouched photographs illustrating the effects produced upon experimental trypanosomiasis of rabbits by treatment with *N*-phenylglycineamide-*p*-arsonic acid. Objects are represented at about two-thirds their natural size, except where otherwise specified.

Figs. 1 to 17 illustrate the effect of single dose treatment upon acute and subacute initial infections.

PLATE 17.

FIG. 1. *Tr. brucei.* Acute infection of 15 days duration. Day of treatment with 0.35 gm. per kilo of body weight given intravenously. There is marked swelling of the face, eyelids, and base of both ears. General condition of animal good.

FIG. 2. 1 week after treatment. Appearance of rabbit normal. Permanently cured.

PLATE 18.

FIG. 3. *Tr. brucei.* Acute infection of 15 days duration. Objects represented at their natural size in Figs. 3 and 4. Day of treatment with 0.2 gm. per kilo of body weight given intravenously. The vulva and anus are markedly swollen and congested and are somewhat indurated. General condition of rabbit is good.

FIG. 4. 5 days after treatment. Rabbit permanently cured.

FIG. 5. *Tr. brucei.* Very severe, acute infection of 23 days duration. Day of treatment with 0.6 gm. per kilo of body weight given intravenously. The face, eyelids, and the lower portions of the ears are extremely swollen and indurated. The general physical condition of the rabbit is fairly good.

PLATE 19.

FIG. 6. 1 week after treatment. Slight residual fullness of the face. The hair over the nose and both upper lids is coming off. Animal's general condition is good.

FIG. 7. 29 days after treatment. Permanently cured.

PLATE 20.

FIG. 8. *Tr. gambiense.* Severe subacute infection of 36 days duration. Objects represented at their natural size in Figs. 8 to 11. Day of treatment with 0.2 gm. per kilo given intravenously. Both testicles are enlarged to about three times normal size and the scrotum is thickened, indurated, and cyanotic; on the right there are three large superficial ulcerations with scab formation. The testicles and cords are also enlarged and indurated. Rabbit's general condition is fairly good.

FIG. 9. 5 days after treatment. Scrotal scabs are separating.

FIG. 10. 19 days after treatment. There is a small residual adherent scab at the tip of the right scrotum. Testicle negative.

FIG. 11. 111 days after treatment—the day rabbit was discarded as permanently cured. External genitalia negative.

PLATE 21.

FIG. 12. *Tr. equiperdum.* Severe subacute infection of 36 days duration. Day of treatment with 0.3 gm. per kilo of body weight given intravenously. Rabbit is very thin and weak. There is marked swelling of the face with moderate involvement of the ears. Both eyes, especially the right, are partially closed and the upper eyelids are swollen and reddened.

PLATE 22.

FIG. 13. 5 days after treatment. There are no signs of trypanosomiasis and the animal's general condition is excellent. Ultimate recurrence.

PLATE 23.

FIG. 14. *Tr. evansi.* Severe subacute infection of 36 days duration. Day of treatment with 0.3 gm. per kilo of body weight given intravenously. Both ears are involved nearly to the tip, the left more than the right; the face is swollen with thickening of the tissues over the bridge of the nose and of the lips. All four eyelids are swollen and reddened; there is a mucopurulent discharge from both eyes, which are almost closed, and numerous yellowish scabs adherent to the lids. General physical condition of rabbit is good.

FIG. 15. 5 days after treatment. Very marked improvement. General condition good.

PLATE 24.

FIG. 16. 15 days after treatment. Scabs over end of nose and lips have desquamated. Beginning growth of hair.

FIG. 17. 19 days after treatment. The hair over the nose and lips is growing rapidly. Rabbit's condition is good with a considerable gain in weight. Permanently cured.

PLATE 25.

Figs. 18 to 23 illustrate the effect of repeated dose therapy upon a chronic initial infection.

FIG. 18. *Tr. brucei.* Extremely severe chronic infection of 48 days duration. Day of beginning treatment with three doses of 0.2 gm. per kilo of body weight repeated at 24 hour intervals and given intravenously. There is a marked and extensive indurated swelling of the tissues of the ears, face, eyelids, nose, and

lips with a purulent exudate from the eyes and nose. Rabbit is weak and emaciated.

FIG. 19. 6 days after treatment had been begun. Marked improvement in the local clinical signs of the infection and in the rabbit's general condition.

PLATE 26.

FIG. 20. 6 days after treatment had been begun.

FIG. 21. 13 days after treatment had been begun.

PLATE 27.

FIG. 22. 21 days after treatment had been begun. There is a very marked growth of hair in the bald areas of the face and ears.

FIG. 23. 40 days after treatment. Permanently cured.

PLATE 28.

Figs. 24 and 25 illustrate the effect of single dose treatment upon an acute relapsed infection.

FIG. 24. *Tr. brucei.* Acute relapse of 7 days duration. Day of treatment with 0.5 gm. of the drug per kilo of body weight given intravenously. The entire left side of the face including the eyelids and ears is swollen. Good general physical condition.

FIG. 25. 1 week after treatment. Rabbit appears entirely normal. Permanently cured.

PLATE 29.

Figs. 26 to 44 illustrate the effect of repeated dose treatment upon acute, subacute, and chronic relapsed infections.

FIG. 26. *Tr. brucei.* Severe acute relapse of 8 days duration. Day of beginning treatment consisting of three doses of 0.15 gm. per kilo of body weight repeated at 24 hour intervals and given intravenously. The face is markedly swollen and indurated and both eyes are closed. The base of both ears is similarly involved. General condition of the rabbit is fair.

FIG. 27. 6 days after treatment had been begun. Slight residual swelling over the bridge of the nose. General condition excellent. Probable permanent cure.

PLATE 30.

FIG. 28. *Tr. brucei.* Severe relapse of 15 days duration on the day of beginning treatment with 0.5 gm. per kilo of body weight given intravenously and repeated 14 days later. There is marked involvement of the face, ears, eyelids, nose, and lips and a mucoserous nasal discharge. Animal very thin and weak.

FIG. 29. 49 days after treatment. Appearance of rabbit normal except for the twisting of the head to the right. Probable permanent cure.

PLATE 31.

FIG. 30. *Tr. brucei.* Severe relapse of 22 days duration on the day of beginning treatment with 0.4 gm. per kilo of body weight given intravenously. The face, base of both ears, eyelids, nose, and upper lips are markedly swollen and edematous and there are thick scabs on the lips.

FIG. 31. 7 days after treatment had been begun. There is a marked improvement in the clinical signs.

PLATE 32.

FIG. 32. 9 days after treatment had been begun. Improvement continues with increase of weight.

FIG. 33. 15 days after treatment had been begun. Clinical signs of the infection have recurred.

PLATE 33.

FIG. 34. 21 days after treatment had been begun. Marked improvement with desquamation of the scabs and necrotic tissue in the involved area. General condition excellent.

FIG. 35. 28 days after treatment had been begun. Further improvement with rapid return to normal appearance. Ultimate relapse.

PLATE 34.

FIG. 36. *Tr. brucei.* Advanced chronic relapse of about 3 months duration on the day of beginning treatment with repeated doses of 0.75 gm. per kilo of body weight given intravenously. There is marked involvement of the face, eyelids, base of ears, and nose with loss of hair and scab formation. Rabbit is weak but not markedly emaciated.

FIG. 37. *Tr. brucei.* Day of treatment. Objects represented at their natural size. The prepuce is markedly swollen and indurated; the testicles and scrotum are very firm and indurated although not enlarged.

PLATE 35.

FIG. 38. 3 days later.

FIGS. 39 and 40. 1 week later. The appearance of the head and the external genitalia is normal except for the absence of hair and a few tiny scabs around the right eye. In Fig. 40 objects are represented at their natural size.

PLATE 36.

FIG. 41. 12 days later. There is a growth of fine hair on the bald patches about the head.

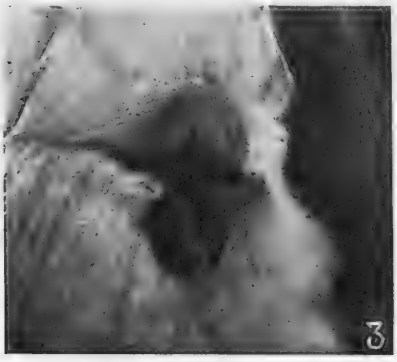
FIG. 42. 17 days later. Rapid growth of hair continues.

PLATE 37.

FIG. 43. 22 days later. Rabbit's appearance is entirely normal except for a tiny bald spot below the inner canthus of the right eye.

FIG. 44. 1 year later. Animal permanently cured.

(Pearce and Brown: Trypanosome and spirochete infections.)



(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)

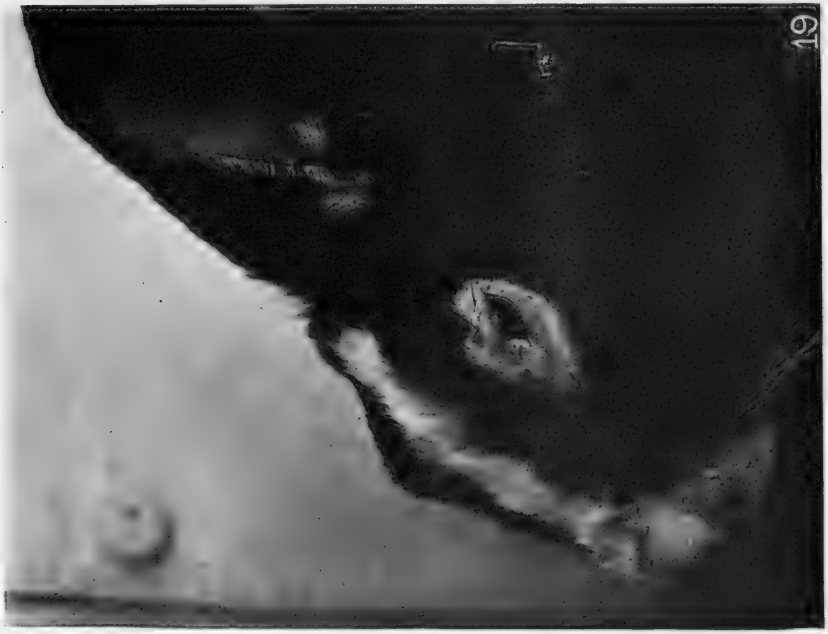
(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)

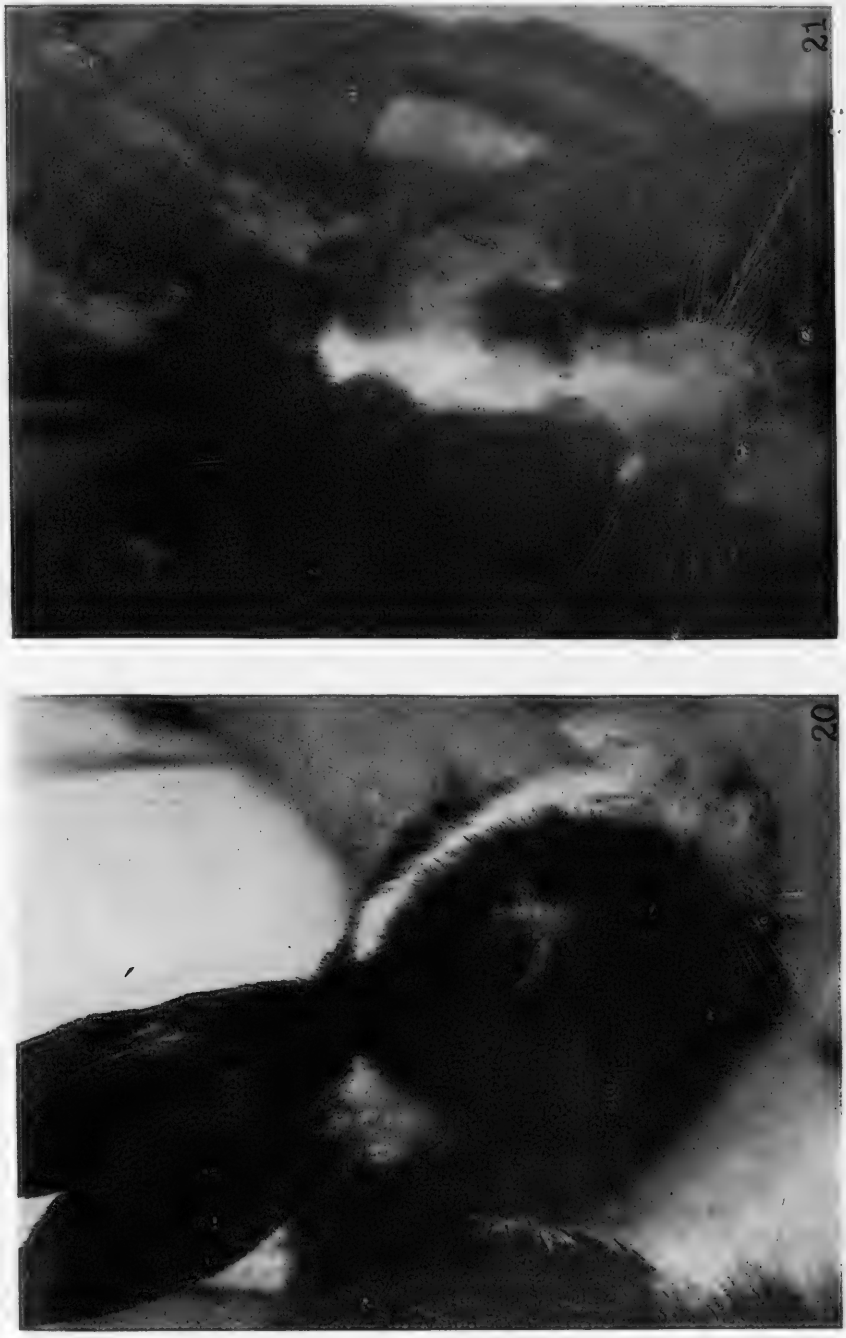
(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)

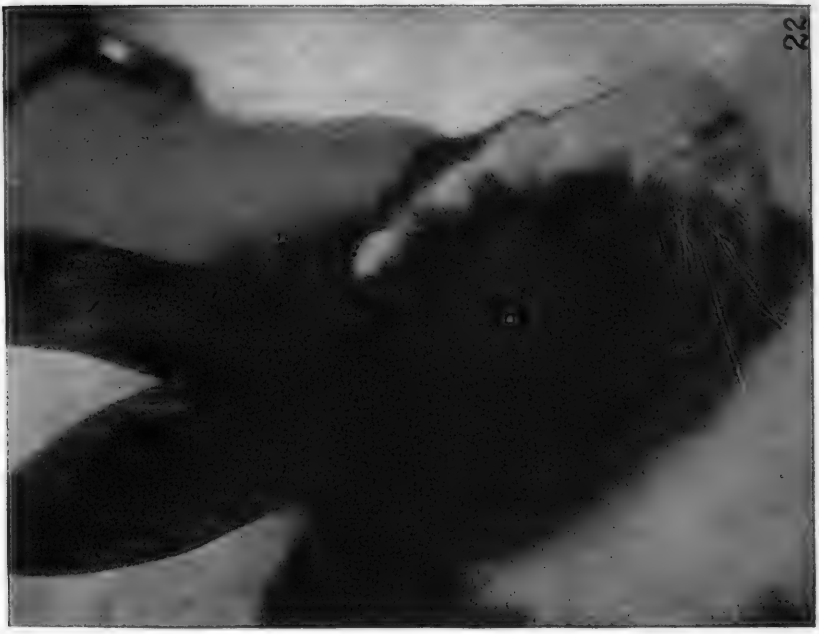
(Pearce and Brown: Trypanosome and spirochete infections.)



(Pearce and Brown: Trypanosome and spirochete infections.)



(Pearce and Brown: Trypanosome and spirochete infections.)



(Pearce and Brown: Trypanosome and spirochete infections.)

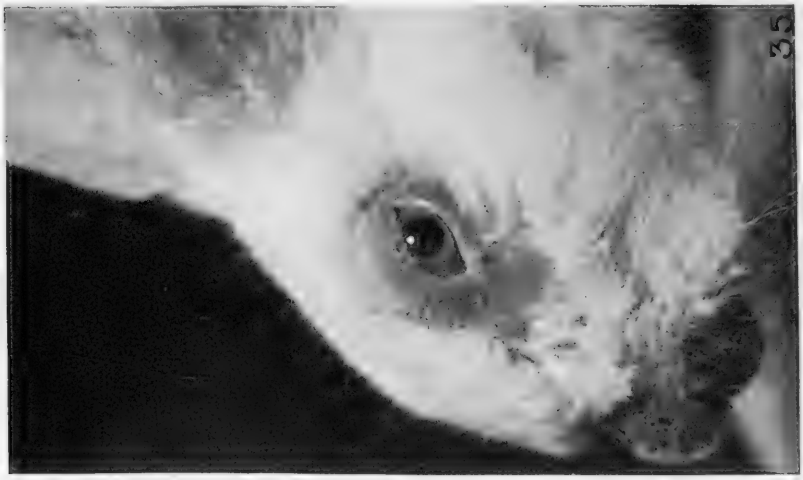
(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)

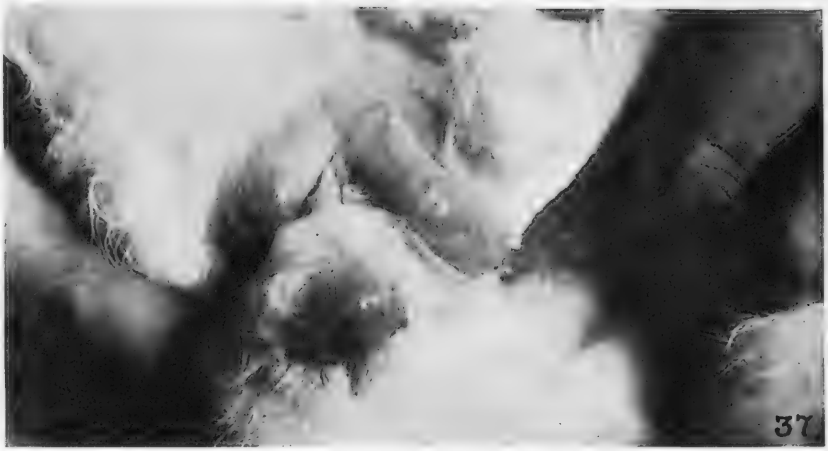
(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)



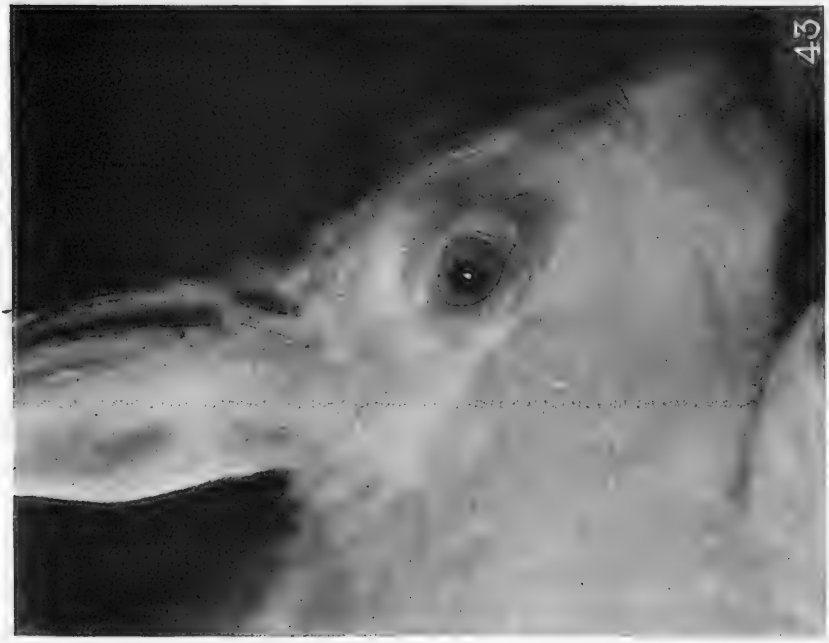
(Pearce and Brown: Trypanosome and spirochete infections.)



(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)

(Pearce and Brown: Trypanosome and spirochete infections.)



(Pearce and Brown: Trypanosome and spirochete infections.)

CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

Biological Series. IV.

The Action of N-Phenylglycineamide-*p*-Arsonic Acid upon Spirochete Infections.

By WADE H. BROWN, M.D., and LOUISE PEARCE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 38 to 44.

(Received for publication, June 18, 1919.)

A second group of conditions upon which the action of *N*-phenylglycineamide-*p*-arsonic acid has been studied is the infections produced in laboratory animals by spirochetes of the *recurrens* group and by *Treponema pallidum*. Early in the course of these studies, it was found that the action of the drug upon these organisms was not so pronounced as in the case of the trypanosomes. Accordingly, the experiments which were carried out were designed more for the purpose of studying the range and character of the action than as attempts to cure the infections.

Blood Spirochetes.

The action of A 63 upon the spirochetes of relapsing fever was studied in both rats and mice infected with *Sp. obermeieri* and with *Sp. novyi*. The animals for these experiments were inoculated intraperitoneally with blood suspensions of the organisms given in such doses as would produce an infection of the desired character—usually an infection which would show a few spirochetes in the peripheral blood within 24 hours after inoculation. The animals were then treated by intraperitoneal injection of single doses of the drug and the results followed from day to day by examination of tail blood under dark-field illumination. Finally, at the end of 60 days, surviving

animals were reinoculated as a means of determining the presence or absence of immunity such as usually develops in the course of untreated infections which terminate in spontaneous recovery, or in treated animals which have not been cured, and is absent in those which have been cured.

The results obtained from the treatment of rats infected with either of the spirochetes mentioned were almost entirely negative. The blood of these animals could not be cleared of spirochetes except by the use of doses of the drug which were nearly always fatal (1.5 gm. per kilo), while smaller doses exercised very little influence upon the course of the infection.

The action of A 63 appeared to better advantage in mice, however, due perhaps to the greater tolerance of these animals for the drug. With an average infection, the dose required to clear the blood of spirochetes temporarily was 1.5 gm. per kilo, or approximately the same as that found to be necessary in the case of rats, but no lasting effect was produced until the dose of the drug used reached upwards of 2 gm. per kilo of mouse, or the largest dose which could be used with safety.

The general character of the therapeutic effects produced may be seen by reference to Table I which shows the results obtained from the treatment of one series of mice with doses of A 63 ranging from 1.5 to 2.25 gm. per kilo. The results of blood examinations are given for the 1st week only with the final result obtained from reinoculation at the end of 60 days.

The infection in these mice might seem to have been a rather severe one since all four controls died within 8 days but the results obtained with the treated mice were of an average character. It will be seen from an examination of this table that the course of the infection was modified in all cases. Usually the progress of the infection was checked rather abruptly and the peripheral blood freed of spirochetes within 24 hours, or by crisis as it were. In a few instances (Mice 6 and 12), this effect was accomplished more slowly; the progress of the infection was first arrested and the infection then terminated by lysis instead of by crisis. In still other animals, the effect of the drug was manifested only by a reduction in the severity of the infection as indicated by the numbers of organisms in the peripheral blood. These effects were not always lasting but were followed in some instances by one or more slight relapses.

When the surviving mice were reinoculated at the end of 60 days, an interesting condition was found in that some mice known to have shown a recurrence of spirochetes in the peripheral blood were as susceptible to reinoculation as those in which no relapse had been observed. While the untreated controls in this experiment were all

TABLE I.

Results Obtained from the Treatment of a 24 Hour Infection of Sp. obermeieri in Mice.

Dose per kilo.	No. of mouse.	Results of blood examinations on days following inoculation and treatment.								Blood on days after reinoculation on 60th day.	
		1	2	3	4	5	7	8	60	2	3
gm.											
2.25	1	+	−	−	−	−	−	−	−	+	++
	2	+	−	−	−	−	−	++	−	+	++
	3	+	−	−	−	−	−	−	D.		
	4	+	−	−	−	−	+	−	"		
	5	+	−	−	−	−	D.				
2.0	6	+	+	+	−	−	−	−	−	−	++
	7	+	−	−	−	−	−	−	−	−	++
	8	+	−	−	−	−	−	−	−	−	++
	9	+	−	−	−	−	−	−	−	−	+
	10	+	−	−	−	−	+	+	−	−	++
1.75	11	+	−	−	−	−	D.				
	12	+	+	+	−	−	+	−	D.		
	13	+	−	−	−	−	++	−	"		
	14	+	−	+	++	−	−	−	"		
	15	+	−	−	−	−	−	−	"		
1.5	16	+	−	−	−	−	++	−	−	−	−
	17	+	+	++	−	−	−	−	−	−	−
	18	+	−	++	−	−	++	++	−	−	−
Controls.	19	+	+++	++++	++++	−	D.				
	20	+	+++	++++	++++	−	"				
	21	+	+++	++++	++++	−	−	D.			
	22	+	+++	++++	++++	−	−	"			

In the tables − indicates no spirochetes found, + few spirochetes present, ++ spirochetes fairly numerous, +++ spirochetes numerous, ++++ spirochetes present in large numbers, D. dead.

dead, the negative results obtained with the mice given only 1.5 gm. of the drug per kilo served as checks against the positive results obtained with the other animals.

Relapse and spontaneous recovery without immunity are almost a paradox, but there are a number of suggestions which occur to one as to how or why such anomalous conditions might arise. Whatever the explanation offered, however, it is obvious that the drug has had its effect even in these relapses and that any immunity developed was either so slight or of such short duration that these mice reacted to reinoculation after 2 months, just as if they had been promptly and completely freed of the spirochetes by the administration of the drug.

In estimating the curative effects of A 63, therefore, one can speak with assurance only of the results of reinoculation. On this basis, it was found that mice infected with *Sp. obermeieri* could be cured with doses of A 63 ranging from 1.5 gm. per kilo of mouse upwards, but with no dose of the drug were we able to obtain cures in more than 75 per cent of the animals treated.

Treponema pallidum.

The action of drugs upon syphilitic infections has usually been studied by the use of some form of the infection which can be produced in rabbits by inoculation with *Treponema pallidum*, and these experimental infections have been used in various ways by different investigators. The lesions of the eye, testicle, and skin have all proved of some value but the scrotal chancre is generally regarded as the most serviceable lesion of the group. The effects produced by drugs upon these infections may be estimated conveniently in three ways: first, by the changes produced in the lesions themselves; second, by the effects upon the organisms present in the lesions; and finally, by the duration or permanence of any changes which may be induced.

In this way, the action of A 63 upon scrotal chancres was studied in a series of twenty-nine rabbits. The lesions were measured with calipers and in most instances were photographed before treatment and at various intervals thereafter in order to determine as accurately as possible the character, extent, and rate of the changes which took place. In like manner, the lesions were aspirated and the fluid was

examined by dark-field illumination before treatment and at intervals such as 1, 3, 5, 7 days, etc., after treatment as a means of following the effects upon the spirochetes. The treated animals were kept under constant observation for such periods of time as the purpose of the experiment demanded, which varied from 1 to 7 months with different animals. For the most part, treatment was carried out by the use of single doses of the drug given intravenously; one series of animals was treated by subcutaneous administration, a second by intramuscular, and a third by the use of repeated small doses of the drug given intravenously. Some study was also made of the action of the drug when given by mouth.

The nature of the results obtained from these experiments may be seen by reference to Table II which contains the abbreviated protocols of twelve rabbits treated by intravenous administration of single doses of A 63 ranging from 0.1 to 0.5 gm. per kilo of body weight.

In order to make these results more intelligible, certain details of the experiments must be supplied. Rabbit 1 of this series was inoculated November 20, 1916. The chancres developed rapidly and at the end of 50 days when treatment was carried out, they measured 2.4 by 2.4 by 1.5 cm. (8.64 cc.) and 2.6 by 2.3 by 1.6 cm. (9.568 cc.) on the right and left respectively. On the day following treatment, the chancres were somewhat edematous, and while a few non-motile spirochetes could still be found, they had disappeared entirely by the end of 48 hours and were never found again. The chancres regressed rapidly for 2 weeks after treatment, when the crusts were torn from the ulcers by the animal. The wounds became secondarily infected and healing was somewhat delayed. The animal was still negative when discarded 100 days after treatment.

The second rabbit of this group requires but little comment. It was an animal with moderately large chancres which reacted to treatment precisely as did Rabbit 1. This animal died from hemorrhagic septicemia 6 days after treatment.

The next two rabbits of the series present an important contrast in the effects produced by the drug. Rabbit 3 was inoculated November 20, 1916, and treated January 9, 1917. At the time of treatment the chancres measured 1.7 by 1.55 by 1.25 cm. (3.294 cc.) on the right and 2.1 by 1.8 by 1.5 cm. (5.67 cc.) on the left. They were markedly

TABLE II.

Results Obtained from the Treatment of Rabbits Infected with T. pallidum.

Dose per kilo.	No. of rabbit.	Testicle.	Chancres at time of treatment.		Results of treatment.						Length of observation.
					Spirochetes.				Chancres.		
			Volume.	No. of spirochetes.	24 hrs.	1 wk.	2 wks.	Recurrence.	Time of healing.	Recurrence.	
gm.			*cc.*						*wks.*		*days*
0.5	1	Right.	8.64	++	−	−	−	No.	5	No.	100
		Left.	9.568	++++	+	−	−				
	2	Right.	5.304	+++	−	D.					
		Left.	6.16	++	−						
0.4	3	Right.	3.294	+++	−	−	−	No.	3	No.	207
		Left.	5.67	++++	−	−	−				
	4	Right.	8.1	++++	−	−	−	Yes.	5	No.	147
		Left.	6.799	+++	+	−	−				
0.3	5	Right.	1.89	++++	−	−	−	No.	3	No.	207
		Left.	3.315	+++	−	−	−				
	6	Right.	3.956	+++	−	−	−	Yes.	3	No.	207
		Left.	2.925	++++	−	−	−				
0.2	7	Right.	1.3	+++	−	−	−	No.	3	No.	85
		Left.	3.22	++++	+	+	−				
	8	Right.	3.47	++++	+	+	−	Yes.	Not healed.	No.	85
		Left.	3.31	++++	+	−	−				
	9	Right.	4.817	++++	+	−	+++		D.		
		Left.	2.755	++++	+	++	+				
	10	Right.		++			+	Never clear.	3	On left.	D.
		Left.	3.27	+++	+++	+	++				
0.1	11	Right.	2.38	+++	+++	−	−	Yes.	2	Yes.	72
		Left.		No lesion.							
	12	Right.	1.586	+++	++	+	++	Never clear.	Not healed.	Yes.	
		Left.	1.42	+++	++		+				

indurated and growing very actively (Fig. 1). Spirochetes were numerous. On aspiration 24 hours later, there was a marked increase in the fluid content of the lesions and no spirochetes could be found nor were they observed at any subsequent examination. The lesions regressed rapidly and at the end of 3 weeks were completely healed with moderate residual thickening in the region of the scars on both sides (Figs. 1 to 4). This rabbit was kept under constant observation for 207 days during which time no lesions, local or general, were observed.

Rabbit 4 was treated 40 days after inoculation. On the day of treatment, the chancres measured 2.5 by 1.8 by 1.8 cm. (8.1 cc.) on the right and 2.1 by 1.85 by 1.75 cm. (6.799 cc.) on the left and were of essentially the same character as those of Rabbit 3 (Fig. 5). For the first 2 weeks, the effects of treatment appeared to be about the same as in Rabbit 3. Regression then proceeded more slowly and the lesions were not completely healed until the end of the 5th week (Figs. 5 to 8), and while there was considerable residual thickening about the scars, no spirochetes could be found. This diffuse thickening gradually diminished, but translucent, glistening, and slightly indurated patches developed at the site of the scars (Fig. 9), and on the 52nd day, numerous actively motile spirochetes were found in these patches. This condition persisted with but slight change for several weeks and then cleared up, as shown in Figs. 9 and 10. The point to be noted here is that although spirochetes were present in the scrotum of this animal at the site of the original lesions, the associated lesions were very slight and showed almost no tendency to growth.

Coming to a still smaller dose of the drug, we again have two animals in which the effects produced were somewhat different. Rabbit 5 was treated 50 days after inoculation. The chancres at this time measured 1.4 by 1.35 by 1 cm. (1.89 cc.) and 1.7 by 1.5 by 1.3 cm. (3.315 cc.) on the right and left respectively. They were both actively growing and well indurated chancres; the one on the right showed a small depressed ulcer, while that on the left was not ulcerated. Spirochetes were numerous on both sides. The results of treatment were essentially the same in all respects as those with Rabbit 3. During 207 days observation, neither spirochetes nor lesions of any kind could be found.

Rabbit 6 was inoculated on November 24, 1916, and treated 46

days later. The chancres in this animal measured 2.15 by 1.6 by 1.15 cm. (3.956 cc.) and 1.95 by 1.5 by 1 cm. (2.925 cc.). Spirochetes disappeared and healing of the chancres took place in the usual manner. There was no sign of relapse for 7 weeks after treatment when several small nodules not more than 1 mm. in diameter appeared in the region of the scar and along the vessels in the dorsal fold of the scrotum on the left. Puncture of these nodules showed numerous actively motile spirochetes. Similar lesions developed later on the right. This animal was kept under close observation for 23 weeks after these lesions appeared, during which time the lesions showed very little change, until they finally regressed and disappeared.

In the next group of rabbits, there were four animals which gave a variety of results. Rabbit 7 responded to treatment by a gradual disappearance of the spirochetes and by rapid regression and healing of the lesions which was almost complete in 3 weeks (Figs. 11 to 14), and remained negative during a period of 85 days observation.

Rabbit 8 also showed a reduction in the spirochetes, and for a short time near the end of the 2nd week after treatment, no spirochetes could be found. In the meantime the chancres had decreased to less than half their original size, but at this point regression ceased. The chancres did not grow again, however, but after remaining stationary for a week or so, underwent spontaneous regression.

Rabbits 9 and 10 behaved still differently. At no time were the lesions of these animals free from spirochetes with the exception of one chancre of No. 9. Nevertheless, the chancres all regressed at a normal rate, and in No. 10, they were healed at the end of 3 weeks with slightly thickened, glistening patches at the site of the scars (Figs. 15 to 18). This rabbit lived only 36 days after treatment, but during this time, there was no renewal of the growth of the lesions, although numerous actively motile spirochetes were present all the while. The other rabbit, No. 9, died of hemorrhagic septicemia before the chancres had healed.

The final group of two rabbits gave results of much the same character as those of the preceding group. Rabbit 11 was inoculated November 1, 1916, but there was very little reaction for 2 months. The chancre on the right then developed rapidly and when treatment was carried out 97 days after inoculation (or about 40 days after the

beginning of the specific reaction) the main chancre mass measured 1.85 by 1.14 by 1.13 cm. (2.38 cc.). On the left, there was only a tiny nodule. Spirochetes disappeared from the chancre in 1 week after treatment and the lesion was healed with moderate residual thickening at the end of 2 weeks (Figs. 19 to 21). The animal was observed for 72 days during which time a small nodule developed in the deeper tissues of the scrotum beneath the scar. A similar nodule also appeared in the left scrotum but neither of these nodules grew and had almost disappeared at the time the animal was discarded.

Rabbit 12 was inoculated December 18, 1916, and treated 49 days later. The chancre on the right measured 1.48 by 1.24 by 0.87 cm. (1.586 cc.) and that on the left 1.41 by 1.2 by 0.84 cm. (1.42 cc.). They were both well indurated and actively growing chancres (Fig. 22). The effect of treatment was to reduce the spirochetes to a moderate extent and to cause considerable regression of the lesions lasting over a period of about 12 days (Figs. 23 and 24). Both chancres then began to increase in size (Fig. 25), in which respect the result differed from that usually obtained from the use of larger doses of the drug.

To the effects which have been described, one other type of reaction should be added; namely, that of the refractory animal or the refractory infection, which was encountered twice in our series of twenty-nine rabbits. The best example which we have of the infection which did not yield to treatment is that shown in Figs. 26 to 29. This animal was inoculated November 8, 1916. For 2 months, the growth of the chancres was rather slow and irregular, but after that they developed very rapidly and measured 1.82 by 1.57 by 1.3 cm. (3.715 cc.) and 1.75 by 1.7 by 1.35 cm. (4.016 cc.) 87 days after inoculation. Both chancres were markedly indurated and contained unusually large numbers of spirochetes. The rabbit was given three intravenous injections of A 63, 48 hours apart, each dose representing 0.1 gm. per kilo. The effect of this treatment was comparatively slight. The spirochetes were very little affected, but the chancres diminished in size for about 9 days with a maximum decrease of 0.4 cm. in all dimensions. They then began to increase in size and 3 weeks after treatment (Fig. 28) measured 1.67 by 1.93 by 0.9 cm. (2.901 cc.) and 1.5 by 1.37 by 0.9 cm. (1.85 cc.). The rabbit was then treated for the second time with a single dose of 0.3 gm. given intramuscularly

with an effect which was but slightly greater than that of the first treatment, the extent of which is shown in Fig. 29.

While the effects which have been described relate almost entirely to intravenous therapy, much the same results were obtained when the drug was given either subcutaneously or intramuscularly. The chief difference noted was that slightly larger doses of the drug were required by either of these routes to produce effects comparable with those obtained by intravenous administration. The drug was found to be active also when given by mouth, but this method of treatment was not used to a sufficient extent to warrant any statement as to its relative value.

Finally, one series of rabbits was treated by the use of repeated small doses of A 63 given intravenously, mainly for the purpose of getting an idea of the size of the dose and the interval between doses which would be required to maintain a continuous therapeutic effect. Without going into the details of these experiments, it may be said that the smallest dose which appeared to exercise a definite influence upon the infection was 0.1 gm. per kilo and that such doses could not be spaced more than 48 hours apart if the effect was to be made continuous. The experiments were not carried far enough to determine just how many such doses would be required to produce a given end-result.

From these experiments one may gain a fair impression of the effects produced by the amide of *N*-phenylglycine-*p*-arsonic acid upon experimental infections of *Treponema pallidum*. As seen by examination of fluid drawn from the lesions, the effect upon the spirochetes is to produce an impairment of motility or a complete loss of motion followed by degeneration and gradual disintegration so that the spirochetes eventually disappear from the lesions if the dose used is sufficiently large. One may be reasonably certain, therefore, that the drug possesses some measure of spirocheticidal action, but the dose required to produce such an effect as that described is comparatively large. At least 0.1 gm. per kilo is necessary to produce any appreciable effect upon the spirochetes; and the effect is usually not very pronounced until the dose used reaches upwards of 0.3 gm. per kilo, and even 0.4 gm. does not always insure a permanent disappearance of the infecting organisms.

The effect of the drug upon the lesions themselves is much more definite and apparently out of proportion to the effect produced upon the spirochetes. As we have seen, complete resolution and healing of scrotal chancres may be accomplished with doses as small as 0.1 gm. per kilo, and this is the usual result obtained with doses as large as 0.2 to 0.3 gm. whether spirochetes are destroyed or not. Further than this, the effects, as far as the lesions are concerned, appear to be more enduring. In our experience, lesions which have once healed, have shown little or no tendency to recur even though actively motile spirochetes were present. In a way, this peculiar type of action reminds one of the process of spontaneous control of *pallida* infections in rabbits; but it is not the same, and whatever the explanation of these effects may be, they are definitely referable to the action of the drug.

CONCLUSIONS.

To summarize the results obtained from these experiments, one may say that *N*-phenylglycineamide-*p*-arsonic acid is capable of exercising a very definite effect upon the course of infections produced by spirochetes of the *recurrens* group and by *Treponema pallidum*. It is more difficult to say, however, just how these effects should be interpreted. In the case of the blood spirochetes, the infection is ameliorated, and even though the spirochetes are not immediately destroyed, the infection is frequently brought to a termination which leaves the animal in a condition not unlike that produced by more powerful spirocheticidal agents. That is, the infecting organisms are either affected in such a way that they eventually die off or are destroyed by the host in such a way that no lasting immunity is developed in consequence of their destruction.

Apparently much the same type of reaction occurs in the treatment of rabbits infected with *Treponema pallidum*. It is possible that when very large doses of the drug are used, these organisms may be completely destroyed, but it is certain that in other cases, where complete healing of the lesions is accomplished as a result of treatment, the organisms are not destroyed. Moreover, it appears that such a result can be accomplished in the presence of numerous actively motile spirochetes, and once the effect of the drug has reached this

point, either the capacity of the spirochetes for stimulating reaction on the part of the tissues is lowered or else the reactivity of the tissues is reduced. At any rate, living spirochetes may remain in the tissues for considerable periods of time without giving rise to the usual tissue reaction which characterizes these infections.

With either group of organisms, therefore, *N*-phenylglycineamide-*p*-arsonic acid appears to act in a manner somewhat different from that of the usual spirocheticidal agents. While it does possess a considerable degree of spirocheticidal action, its chief effect is seen in the peculiar manner in which it modifies or controls the course of these infections.

Further than this, we shall not attempt to go at present. As set forth in the literature, the facts and principles upon which such experiments depend are so few as to offer almost no basis for an interpretation of these experiments. When we have had an opportunity of reporting our own experience in dealing with these infections, we may return to a consideration of the facts here reported.

EXPLANATION OF PLATES.

The figures are reproductions of untouched photographs illustrating the effects produced upon scrotal chancres of rabbits by treatment with *N*-phenylglycineamide-*p*-arsonic acid. Objects are represented at their natural size.

PLATE 38.

FIGS. 1 to 4. Rabbit 3. Effect produced by 0.4 gm. per kilo of body weight.

FIG. 1. Chancres at time of treatment.

FIG. 2. 1 week after treatment.

FIG. 3. 2 weeks after treatment.

FIG. 4. 3 weeks after treatment. The lesions are healed with some residual thickening especially noticeable on the right, but no spirochetes could be found.

PLATE 39.

FIGS. 5 to 10. Rabbit 4. A peculiar therapeutic effect following the administration of a dose of 0.4 gm. per kilo.

FIG. 5. Chancres at the time of treatment.

FIG. 6. Chancres regressing rapidly.

FIG. 7. 4 weeks after treatment. Chancres unhealed but still regressing slowly.

FIG. 8. 5 weeks after treatment. Chancres are healed but the scars are thickened and translucent, especially that on the right.

FIG. 9. 9 weeks after treatment. The lesions are still healed but scars are now represented by translucent, highly refractile plaques which contain numerous actively motile spirochetes.

FIG. 10. 147 days after treatment. The foci of infection have cleared spontaneously and the scrotum is normal.

PLATE 40.

FIGS. 11 to 14. Rabbit 7. The effect of a single dose of 0.2 gm. per kilo.

FIG. 11. Chancres at the time of treatment. That on the right is an unulcerated globular mass.

FIG. 12. 1 week after treatment; very rapid regression of the lesions.

FIG. 13. 3 weeks after treatment. The chancre on the right has entirely disappeared; that on the left still shows a small thickened mass.

FIG. 14. 4 weeks after treatment. Resolution complete.

PLATE 41.

FIGS. 15 to 18. Rabbit 10. An instance of the healing of chancres with a single dose of 0.2 gm. per kilo while actively motile spirochetes were constantly present.

FIG. 15. Chancres at the time of treatment.

FIG. 16. 1 week after treatment; marked regression of the lesions.

FIG. 17. 2 weeks after treatment.

FIG. 18. 3 weeks after treatment. The lesions have practically disappeared, but actively motile spirochetes are fairly numerous in the scars of both sides. There was a slight recurrent patch in the left scrotum.

PLATE 42.

FIGS. 19 to 21. Rabbit 11. An effect which may be accomplished by a dose of 0.1 gm. in lesions which are easily influenced.

FIG. 19. Chancre at the time of treatment.

FIG. 20. 1 week after treatment.

FIG. 21. 2 weeks after treatment.

PLATE 43.

FIGS. 22 to 25. Rabbit 12. The extent of the effect usually produced by a dose of 0.1 gm. per kilo.

FIG. 22. Chancres at the time of treatment.

FIG. 23. 8 days after treatment.

FIG. 24. 2 weeks after treatment. The chancre on the left is healed, but there is a considerable mass of unresolved tissue in the scrotum; that on the right is increasing.

FIG. 25. 3 weeks later. Both chancres growing actively.

PLATE 44.

FIGS. 26 to 29. Chancres which proved refractory to treatment with this drug.

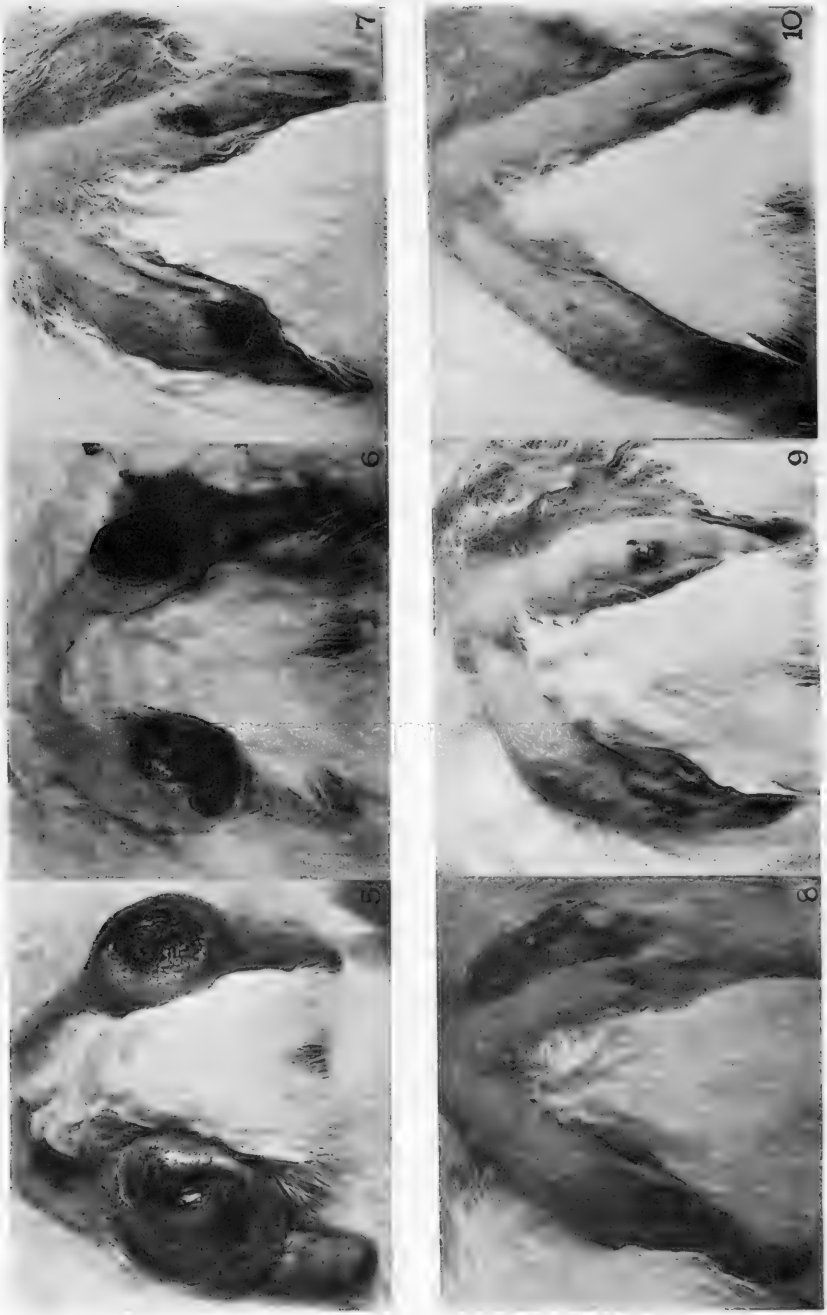
FIG. 26. Chancres at the time of first treatment which consisted of three doses of 0.1 gm. per kilo given intravenously at intervals of 48 hours.

FIG. 27. 1 week after treatment had been commenced; practically the full extent of the reduction of the lesions is shown.

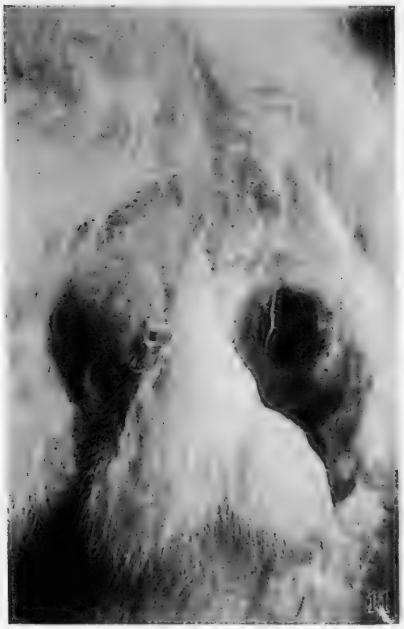
FIG. 28. 2 weeks later; the growth of the chancres in the interim is shown. Animal retreated with 0.3 gm. per kilo given intramuscularly.

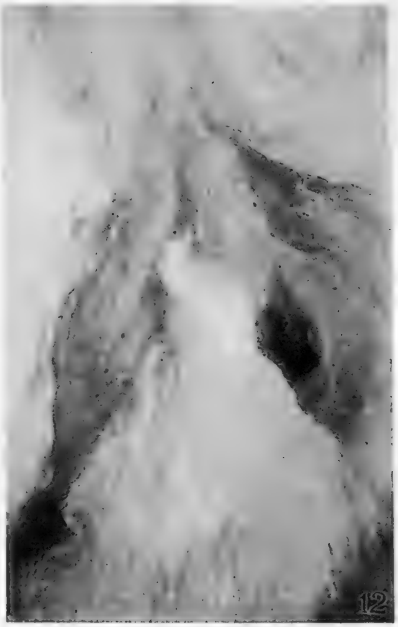
FIG. 29. 1 week later; a decided effect is shown, but renewed activity set in almost immediately.

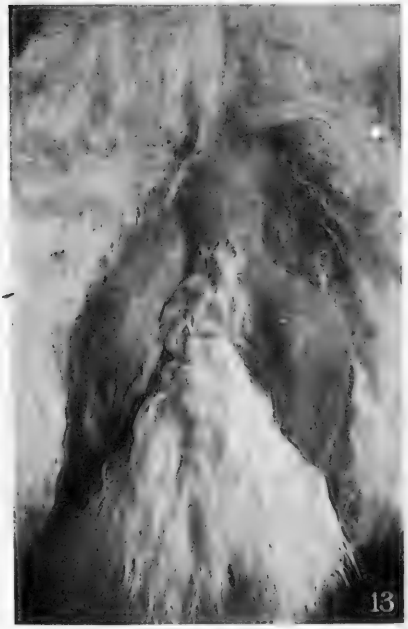
(Brown and Pearce: Trypanosome and spirochete infections.)

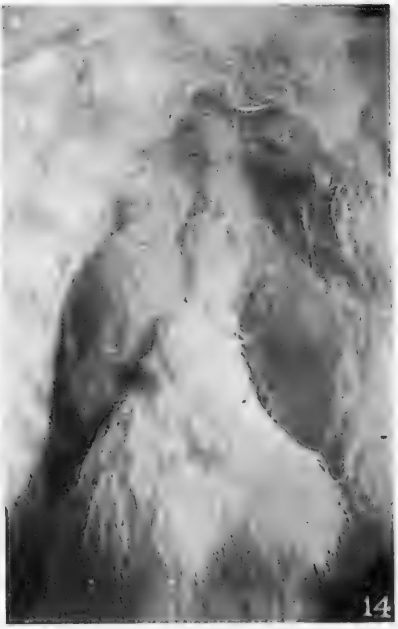


(Brown and Pearce: Trypanosome and spirochete infections.)

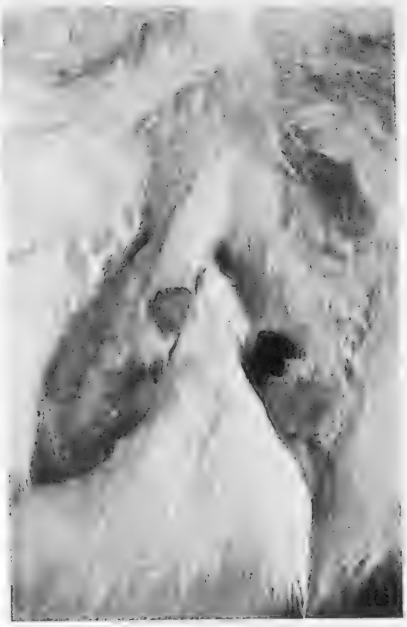


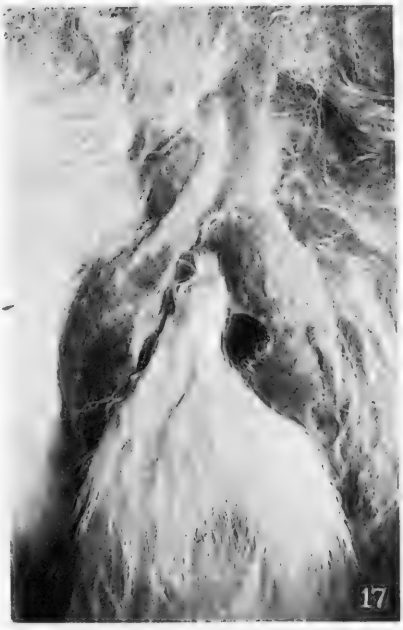


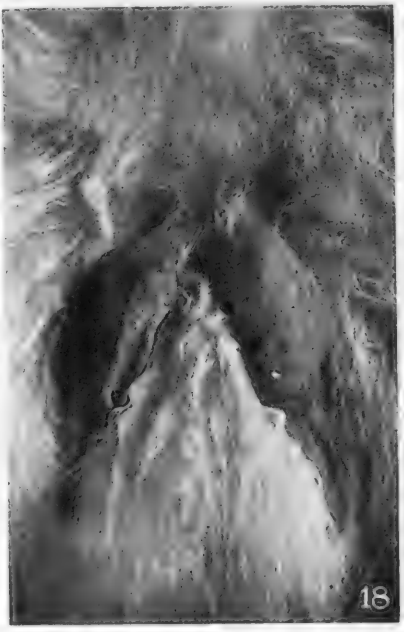




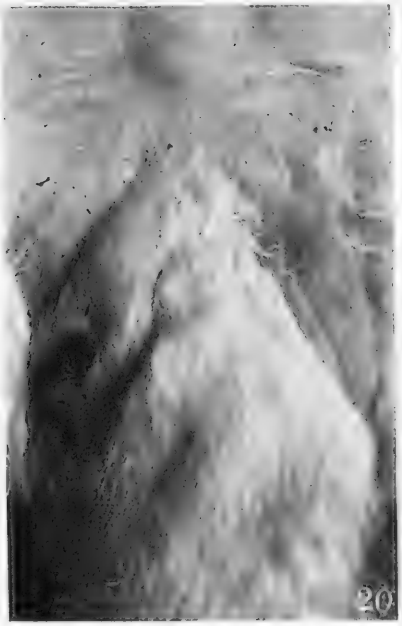
(Brown and Pearce: Trypanosome and spirochete infections.)



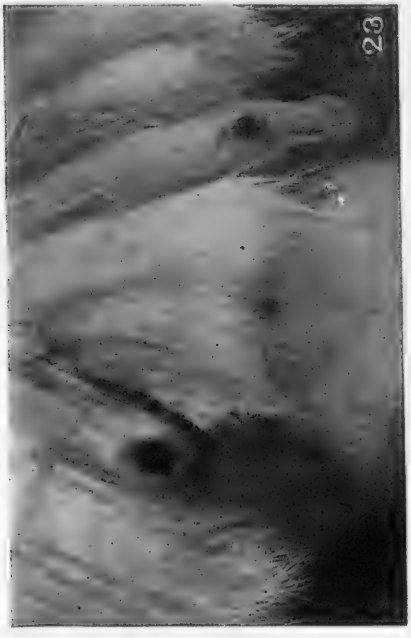


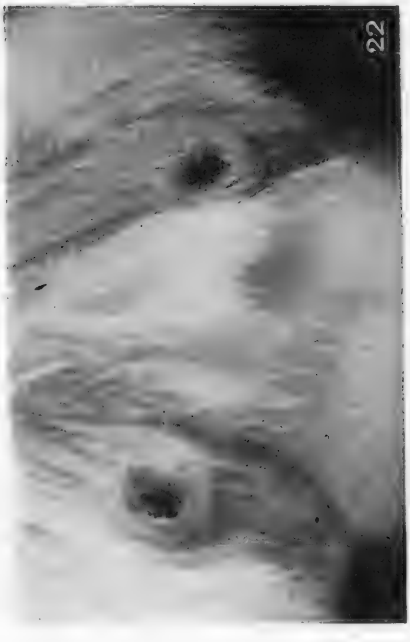


(Brown and Pearce: Trypanosome and spirochete infections.)



(Brown and Pearce: Trypanosome and spirochete infections.)

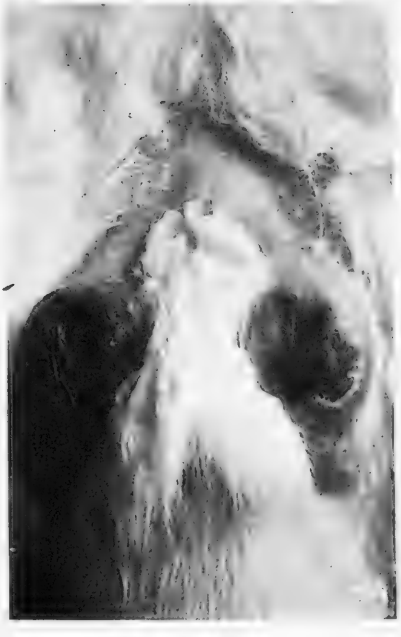


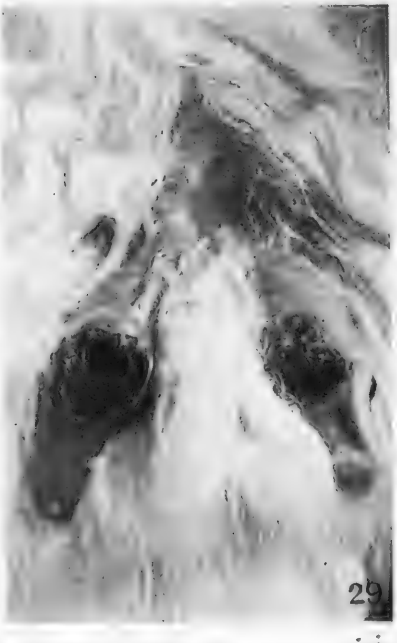


(Brown and Pearce: Trypanosome and spirochete infections.)

26

27




29

THE OCCURRENCE OF BACILLUS INFLUENZÆ IN THE NORMAL THROAT.

By AGNES I. WINCHELL and ERNEST G. STILLMAN, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, July 15, 1919.)

The present study is a continuation of the work of Pritchett and Stillman on the occurrence of *Bacillus influenzæ* in throats and saliva, with additional observations upon the media used and a study of the distribution of *Bacillus influenzæ* among the personnel of two institutions.

Pritchett and Stillman[1] found that of 177 persons who gave no history of having had influenza, 74, or 42 per cent, harbored *B. influenzæ* in their throats during November and December of 1918. The total incidence of influenza bacillus carriers among 231 normal and late convalescent individuals was 99, or 43 per cent. From 49 cases of uncomplicated influenza, *B. influenzæ* was recovered in 41 instances, or 83 per cent, while from 43 cases complicated by bronchopneumonia this organism was cultivated in 40, or 93 per cent. Of 6 cases of bronchopneumonia, which were probably late cases of influenza, all showed influenza bacilli. The incidence of *B. influenzæ* was much lower in 20 cases of lobar pneumonia, as only 11 cases, or 55 per cent, were positive. Pneumococci of Types III and IV, which are the groups usually encountered in normal mouths, were the types of pneumococci which were recovered from the cases of influenza complicated with bronchopneumonia.

Methods.

As in the previous study, the medium used in this investigation for the isolation of *Bacillus influenzæ* was Avery's[2] oleate hemoglobin agar. 5 cc. of a 2 per cent solution of neutral sodium oleate were added to 95 cc. of meat infusion agar which made a final concentration of oleate of 1: 1,000. 1 cc. of a suspension of rabbit red blood cells was then added while the agar was still hot. Plates were poured

[1] Pritchett, I. W., and Stillman, E. G., *J. Exp. Med.*, 1919, xxix, 259.

[2] Avery, O. T., *J. Am. Med. Assn.*, 1918, lxxi, 2050.

containing about 15 cc. each. The medium was made fresh each day as the surface soon became dry.

The optimum hydrogen ion concentration for the isolation of influenza bacillus from the throat seems to be between pH 7.2 and 7.5. In every test of medium which was more alkaline than that having a pH of 7.5, unsatisfactory results were obtained. In one instance cultures were made on agar at pH 7.9 from 12 individuals who previously had been persistent carriers. Though the cultures were repeated, only 2 showed small colonies of *Bacillus influenzæ*. But, when repeated a third time on medium with a pH of 7.5, 8 were found

TABLE I.

Comparison of Cultures upon Media of Different Hydrogen Ion Concentrations.

Culture No.	pH 8.0		pH 7.4	
	Series 1.	Series 2.	Series 1.	Series 2.
1	−	−	++	++
2	−	−	++	++
3	−	−	−	−
4	−	−	−	−
5	−	+	++	+
6	−	−	+	++
7	−	−	++	+
8	−	+	++	+
9	−	−	++	++
10	−	−	−	++
Total positive.	0	2	7	8

+ indicates colonies of *B. influenzæ* present; ++ many colonies of *B. influenzæ* present.

to be carrying influenza bacilli. Of 36 other individuals chosen at random, 14, or 38 per cent, were positive when agar at pH 7.4 was used, but only 4, or 11 per cent, with agar at pH 7.9. In Table I are given the results obtained in an examination of 10 individuals from whom both positive and negative plates had previously been obtained. Cultures were taken twice during the same day upon medium at pH 8.0 and at pH 7.4. No positive plates were found in Series 1 on medium with a pH of 8.0, but in Series 2, 2 plates contained several small colonies. On the other hand, Series 1 with 7.4 pH agar shows 7 positive plates, and Series 2, 8 positive plates.

Negative results are so consistently obtained with medium having a reaction above pH 7.5 that one is led to question the value of all negative results obtained upon medium with an unknown hydrogen ion concentration.

All cultures here reported, unless otherwise stated, were taken from the posterior wall of the pharynx. As shown by Table II cultures from the tonsils proved to be unsatisfactory. In a series of 77 individuals, 3 cultures were made from the throat—1 culture from each tonsil, and 1 from the posterior wall of the pharynx. In 4 individuals positive cultures were obtained on plates from all three sources. In 4 other individuals the cultures from the pharynx and from one or the other tonsil were positive. In 11 individuals only the culture from

TABLE II.

Comparison of Cultures from the Pharynx and the Tonsils.

Source of cultures.	No. positive.
Pharynx and both tonsils	4
" " one tonsil	4
Only tonsil	0
" pharynx	11
Total No. of cases positive	19
" " " " negative	58
" " " " examined	77

the pharynx showed a growth of influenza bacilli. In no case were the organisms isolated from the tonsil and not from the pharynx. The influenza bacillus can unquestionably be isolated from the tonsils, but as there are so many other colonies on the plates made from the tonsils, the influenza bacilli are either overgrown or the colonies are so small that they are hard to detect. West tubes used in a few instances did not give any more satisfactory results than did the simpler method of pharyngeal cultures. Hence, it seemed unnecessary to subject the large group of individuals studied to the discomfort of nasopharyngeal cultures.

All organisms designated as *Bacillus influenzæ* were demonstrated to possess the typical morphological and cultural characteristics of this organism; all cultures were proved to be definitely hemoglo-

binophilic. With the exception of staphylococci and diphtheroids, only Gram-negative organisms were encountered upon the oleate hemoglobin agar plates. In addition to the influenza bacillus, there appeared on these plates various types of Gram-negative cocci of the *Micrococcus catarrhalis* group and Friedländer's bacillus group, and also the unidentified bacillus, described by Pritchett and Stillman, which is differentiated from *Bacillus influenzæ* by its hemolytic properties.

RESULTS.

Cultures were taken once a month for a period of 6 months from the throats of the personnel of the Laboratories and the Hospital of

TABLE III.

Incidence of B. influenzæ in Convalescent and Normal Individuals.

	Nov. and Dec., 1918.			Jan., 1919.			Feb.			Mar.			Apr.			May.		
	No.	Positive cases.		No.	Positive cases.		No.	Positive cases.		No.	Positive cases.		No.	Positive cases.		No.	Positive cases.	
		No.	Per cent.		No.	Per cent.		No.	Per cent.		No.	Per cent.		No.	Per cent.		No.	Per cent.
Normal individuals	177	74	42	127	17	13	122	42	34	109	56	51	96	33	34	86	34	39
Convalescents	54	25	46	42	1	2	40	16	40	42	19	45	38	15	39	28	13	46
Total	231	99	43	169	18	11	162	58	36	151	75	50	134	48	36	114	47	41

The Rockefeller Institute in accordance with the method outlined above. It has been possible to follow but 84 of the original 231 individuals used in the earlier study. Of these, 15 have never yielded a positive result. 9 of 35 individuals positive upon the original examination have never since shown the presence of *Bacillus influenzæ* in the throat. Of the total 84, 6 have been positive for 5 months and 13 for 4 months.

During the months from December, 1918, to June, 1919, as shown in Table III, the percentage of carriers in a group of approximately 150 individuals has been 43, 11, 36, 50, 36, and 41, respectively. In the previous study the individuals who gave a history of having had

influenza during the time of the epidemic were classed as convalescents. These same individuals have been followed during the complete period. 46 per cent were positive in November and December, 1918, 2 per cent in January, 1919, 40 per cent in February, 45 per cent in March, 39 per cent in April, and 46 per cent in May.

As previously mentioned, any culture medium which varies from the optimum hydrogen ion concentration is unsuitable for use in the isolation of *Bacillus influenzæ* from the throat. The reaction of the medium used in November and December had a pH of 7.2, while that for March, April, and May had a pH of 7.4 and 7.5. The reaction of the medium employed during January and February was not tested, and it is apparent, therefore, that no importance can be attached to the low incidence during January.

TABLE IV.

Incidence of B. influenzæ in Cases of Acute Respiratory Infections.

Date.	No.	Positive.	Per cent positive.
1919			
Feb.	9	5	55
Mar.	16	13	81
Apr.	12	8	67
Total.	37	26	70

As is seen from Table IV, *Bacillus influenzæ* was recovered from 70 per cent of the persons suffering from acute respiratory diseases who were admitted to the Hospital of The Rockefeller Institute during the months of February to April. Thus it is seen that during the same months a larger percentage of positive cultures was obtained from patients suffering from acute respiratory diseases than from normal individuals.

During the latter part of January, an epidemic of influenza occurred in a girls' school. While the pandemic of influenza raged in the country surrounding this institution during the fall of 1918, a strict quarantine was maintained and only a few questionable cases developed. While the institution was still under strict quarantine, a teacher who had spent Sunday at home developed influenza. The

first cases developed in her classes, and within 10 days over half the students were ill. The epidemic then subsided as suddenly as it arose. On February 16 throat cultures were taken from 52 girls who were still in the infirmary. Of these, 20, or 38 per cent, showed *Bacillus influenzæ*. In one cottage which had been under quarantine on account of trachoma since October, 1918, no cases of influenza developed. Of the 20 girls in this cottage, 5, or 25 per cent, showed *Bacillus influenzæ*.

In a boys' orphan asylum where a lax quarantine had been maintained and which gave no history of an epidemic of influenza, 190 throat cultures were taken from boys whose ages varied from 10 to 12 years. *Bacillus influenzæ* was recovered from 74, or 39 per cent.

DISCUSSION.

Whatever may be the etiologic relationship of *Bacillus influenzæ* to epidemic influenza, there is little doubt of the significance of this organism as a secondary invader in this type of respiratory infection. Facts, therefore, concerning the distribution, occurrence, and persistence of *Bacillus influenzæ* in the secretions of the respiratory tract are of importance in epidemiological investigation and in prophylaxis. In a study of the occurrence of *Bacillus influenzæ* in throats and saliva during the epidemic of influenza last fall, Pritchett and Stillman found this organism present in 42 per cent of 177 healthy persons from whom no history of respiratory infection was obtainable. These observers found the same organism in the throats of convalescents from influenza in 46 per cent of individuals studied. In the same epidemic period Lord, Scott, and Nye,[3] by cultural methods, demonstrated influenza bacilli to be present in the pharyngeal secretions of 76 per cent of 34 healthy men of the Harvard Student Army Training Corps. Opie and his collaborators,[4] by cultural and mouse inoculation methods, found *Bacillus influenzæ* in the mouths of 35.1 per cent of all healthy men examined at Camp Funston.

[3] Lord, F. T., Scott, A. C., Jr., and Nye, R. N., *J. Am. Med. Assn.*, 1919, lxxii, 188.

[4] Opie, E. L., Freeman, A. W., Blake, F. G., Small, J. C., and Rivers, T. M., *J. Am. Med. Assn.*, 1919, lxxii, 108.

These figures serve to indicate the wide distribution and prevalence of the organism during the severe epidemic of this acute respiratory disease.

Since the group of individuals studied by Pritchett and Stillman comprised for the most part the personnel of The Rockefeller Institute, it has been possible to make repeated cultural examinations of the throats of 84 of the same persons during the 6 months subsequent to the original observation. From this study certain facts of interest have been acquired concerning the duration of the carrier state, and the relative frequency of this organism in normal throats after the subsidence of the epidemic. The present study indicates that the percentage incidence of those harboring *Bacillus influenzæ* in the upper respiratory tract is as great during the postepidemic period as it was during the influenzal epidemic. During December, 1918, to June, 1919, the percentage of carriers in a group of 150 individuals has averaged 41 per cent per month. This percentage incidence of healthy persons found to harbor influenza bacilli in their throats and saliva is approximately the same as that recorded by Pritchett and Stillman during the height of the epidemic. In addition, it is of interest that in a boys' orphan asylum in which no case of influenza had occurred during the epidemic, 39 per cent of throat cultures taken from 190 boys showed the presence of *Bacillus influenzæ*. This percentage incidence of positive cultures is the same as that found in the examination of 52 convalescents from influenza in an institution for girls in which over half the personnel had suffered from the disease.

Furthermore, it is evident that *Bacillus influenzæ* may persist in the throats of healthy carriers for a considerable period of time. In 6 instances individuals have had positive cultures on repeated examinations during 5 months, and in 13 other instances during a period of 4 months. In chronic non-tuberculous disease of the lungs *Bacillus influenzæ* is known to persist for long periods of time in the secretions of the respiratory tract. The present study, however, demonstrates that in the absence of a focus of infection this bacillus may dwell on apparently normal mucous membranes for long intervals. Whether the organisms thus encountered represent a variety less parasitic than strains actually associated with pathologic lesions

is a problem requiring greater knowledge concerning the biologic differentiation of bacilli of the hemophilic group than is now available.

The technical difficulties incident to the isolation of *Bacillus influenzæ*, particularly from the mixed flora of the upper respiratory tract, have been materially lessened by the use of oleate hemoglobin agar. The optimum hydrogen ion concentration for this medium is shown to lie between pH 7.2 and 7.5. Any variation beyond this range, particularly on the alkaline side, reduces markedly the suitability of this medium for growth of *Bacillus influenzæ*. Oleate hemoglobin agar not only enhances the growth of the influenza bacillus, but by inhibiting the growth of other organisms, such as streptococci and pneumococci, greatly facilitates the isolation of *Bacillus influenzæ* from a focus which harbors a wide variety of other bacteria.

CONCLUSIONS.

1. The frequency of occurrence of *Bacillus influenzæ* in the throats of normal individuals has been as great in the period subsequent to the epidemic of influenza as it was during the period of the epidemic.

2. *Bacillus influenzæ* may persist in the throats of healthy carriers for a considerable period of time.

3. A considerable proportion of individuals not exposed during the epidemic has been found to carry *Bacillus influenzæ* in their throats.

4. Oleate hemoglobin agar has been found to be a very satisfactory selective medium for cultivation of *Bacillus influenzæ* from the throat. To give optimum results it should have a reaction of from pH 7.2 to 7.5.

THE PATHOLOGICAL CHANGES IN THE TESTES IN EPIDEMIC PNEUMONIA.

By RALPH G. MILLS, M. D.

(*From the Department of Pathology of the Johns Hopkins University, Baltimore.*)

Plates 45 to 50.

(Received for publication, July 12, 1919.)

Clinically orchitis or any other affection of the testes is uncommon in primary pneumonia or in pneumonia following either measles or influenza. Osler and McCrae (1) make no mention of this condition as following pneumonia. Equally rare is the atrophy of the testis following recovery from the disease, hence any extensive or permanent injury would be unlikely.

The only reference in literature to changes in the testis following pneumonia is an article by Cordes (2) in which he tabulates the findings in twenty cases of primary pneumonia, in patients between the ages of 21 and 60 years. No case was seen in which the spermatogonia were so badly damaged as to suggest that had the patient recovered there would have been no regeneration of these cells and restoration of function.

Sixty cases were studied. Pneumonia was the cause of death in every instance, but in some cases it was the sole factor, while in others it was preceded by measles or epidemic influenza. The changes in the body directly due to measles and influenza are little known and hence the extent of their influence in the present study must be determined by the indirect method of comparing the picture seen in primary pneumonia with that in pneumonia following measles and influenza. The presence of respiratory irritation and of lowered resistance to infection is well recognized and their importance in a given case becomes manifest with the advent of pneumonia or the awakening of some latent infection.

The bacteriology of the cases varied greatly. The presence and the importance of the hemolytic streptococcus in the first epidemic were generally recognized, but the part played by the Pfeiffer bacillus has been the subject of much discussion. Its presence was frequently noted in the measles epidemic, but no special attention was given it other than to consider it a secondary invader. It attracted attention during the influenza epidemic and the significance of its presence has been much studied.

During these epidemics lobar pneumonia caused by the pneumococcus has been largely replaced by other forms. A single organism was in some cases the apparent cause of the pneumonia, but in many instances two or more of the pathogenic bacteria were associated, the variety of combinations contributing greatly to the complexity of the problem. These cases may be classified into several groups on the basis of the etiological factors involved: (1) primary pneumonia—pneumonia occurring during the measles epidemic and due to the same organisms, but not preceded by an attack of that disease; (2) pneumonia following measles; (3) pneumonia following epidemic influenza. These have been further subdivided according to the occurrence of *Streptococcus hæmolyticus*, *Bacillus influenzæ*, and the pneumococcus, both alone and in combination.

Complete tables of the findings in the cases, based on this grouping, have been made and are given below. The bacteriological findings in these cases are more complete than is usually found. Careful attention was given to the examinations and every effort was made to isolate and identify all organisms present.

With the development of the MacCallum stain (3), by which the Gram-negative organisms may also be identified in sections, the influenza bacillus has been added to several of these cases in which it was not found at previous examinations. In spite of all care it is more than likely that the findings do not report all the organisms present.

It was manifestly impossible to distinguish and study the effect of every organism present; hence the three supposed to be the most important were selected as the basis for comparison. In regard to the general infection and the etiology of the principal lesions, MacCallum (4, 5) has reached certain conclusions concerning the relative importance of the various organisms present in the different cases, and these have been followed in the present study.

The subjects were all soldiers in good health, recently passed through the rigid examination for admission to the Army.[1] The ages varied from 18 to 40 years. Presumably all individuals with any sort of chronic or debilitating disease had been excluded from the Army and the supposition is warranted that these men were in excellent health. This supposition is important in the light of the work of Weichselbaum and Kyrle (6) who have described changes in the testes in persons dying of chronic alcoholism, and of others who mention the effect of tuberculosis, syphilis, liver disease, and malignancy in causing somewhat similar lesions.

Syphilis is always an uncertain factor, but the age of the men, the absence of postmortem luetic signs, the nature of the Army entrance examination, and the continued medical supervision make this disease of less importance in this series than it would be in almost any other that could be secured.

Tuberculosis as an active process is almost negligible, those individuals with any but the most latent lesions having been previously excluded. Military training would have tended to render quiescent any latent infection or to lead to the subsequent elimination of the individual as unfit. In this series cavity formation was found in one case, but this was of such a nature that it had probably not been injurious for any considerable period of time. Presumably the usual number of healed tubercles was encountered that occurs in autopsies on persons dying of other diseases and hence are not considered to be of any importance in the present discussion. It is reasonably safe also to eliminate from this discussion any uncertainty referable to the possible insidious influence of diabetes, liver and kidney alterations, and all other chronic diseases.

The associated organisms were commonly staphylococcus and *B. mucosus capsulatus*. Neither of these is supposed to have been operative to an extent sufficient to contribute much to the pathological picture because of the probability that they were secondary invaders shortly before or possibly after death.

The length of time that the individuals had been in the hospital was variable. The anatomical diagnosis indicates the degree to which other related conditions prolonged or complicated the original picture

[1] The material studied was obtained by Professor W. G. MacCallum at Camps Sam Houston, Dodge, Dix, and Lee during the measles epidemic of 1917 and the influenza epidemic of 1918. The numbers of the autopsies to which reference is made agree with those used in his monograph published by The Rockefeller Institute for Medical Research (4) and in another to be published in the *Johns Hopkins Hospital Reports*, and are here repeated for convenience in making cross-reference.

of the pneumonia. Unfortunately the number of days in the hospital in some cases does not include the length of time covered by the primary disease. In a given instance this fact can be surmised by comparison with others of similar pathological picture. Roughly the severity of the injury corresponds directly with the duration of the pneumonia as closely as with any other single variable factor.

General Description of the Testicular Injury in Pneumonia.

Study of the data presented fails to reveal any special change characteristic of pneumonia that is produced by a particular organism or that follows any antecedent infectious disease or sequel thereto. Essentially the spermatogenetic function is inhibited and the spermatogonia cease their mitotic activity. They apparently become quiescent while the spermatocytes and other derived cells continue for a time under the influence of the mitotic activation received. This activity is only temporary, for definite signs of degeneration soon appear.

At the same time that nuclear and protoplasmic changes are occurring in the derived cells, the protoplasm of the Sertoli cells is undergoing transformation. It begins to break up, loosening the cells held in its syncytium-like meshes with the result that desquamation is very extensive. Some cells still remain attached by protoplasmic threads, and the free edge of the germinal epithelium appears ragged and frayed. The clusters of spermatids about the Sertoli cells are broken up so that imperfectly formed spermatozoa are added to the collection of cellular debris. Fragmentation of the epithelium and disintegration of all dislodged cells proceed fairly rapidly until the tubule is reduced to a condition similar to that found in childhood and cryptorchidism, in which only the spermatogonia and Sertoli cells remain (Fig. 1). In a few instances the spermatogonia also were caused to disappear, leaving the Sertoli cells alone. Further stages of cellular reduction are not seen without concomitant change in the basement membrane.

Alteration of the basement membrane may begin quite early in the process. Invariably this involves the inner hyaline layer which increases in thickness, and as this occurs and as the tubules shrink, per-

haps from loss of cells, it is thrown into folds which more or less disturb the relation of the spermatogonia to the wall. A moderate amount of thickening of the hyaline layer, perhaps up to three or four times its original thickness, may accompany cellular changes of the severity mentioned in the preceding paragraph, but more extensive changes have been seen only with greater reduction in the cellular elements.

The Sertoli cells usually show no evidence of marked alteration. The nuclei are frequently wrinkled or shrunken to a triangular shape, and a nucleolus can usually be distinguished. They occupy the second row in the germinal epithelial layer and assume a palisade arrangement as the rows of cells on either side disappear (Fig. 2). The protoplasm has the appearance of a fine interlacing network after all the cells are gone. An unusual form of protoplasmic alteration is illustrated by Figs. 3 and 4.

Spermatogonia present as a rule a uniformly normal appearance. In the extreme cases the nuclei are darker, more dense, and may be either larger or smaller. Occasionally a chromatolyzed nucleus can be seen and vacant spaces here and there may indicate complete destruction. Plication of the hyaline basement membrane frequently disarranges the uniformity of their distribution.

The spermatocytes by reason of their size, number, and general prominence exhibit distinct signs of injury. The coiled and interlacing chromatin threads tend to become granular and to break up into coarse darkly stained fragments. These have a tendency to fuse but gradually deteriorate, lose their deep color, and soon appear as mere clusters of granules, perhaps for a time presenting a crescentic appearance, but eventually disappearing entirely. These cells are often too deeply buried in the epithelial wall to break loose or to form any considerable part of the central mass of cellular debris. They are almost invariably decreased and in some instances completely absent, affording convincing evidence that the supply of new cells was cut off and that those already formed are in the process of dissolution.

Spermatids are seen in about half the sections, but are not present in half the tubules of any one section. All are loosened and evidently undergoing degeneration. There have been swelling and fusion of the chromatin, reducing the nuclei to homogeneous deeply stained

masses which are partly clinging to the walls and partly mingled with other loosened cells.

The spermatozoa are quite resistant and are relatively slow in disappearing. In most instances the head has retained its characteristic shape but no tails are to be seen. They are usually widely scattered and have not retained the normal relation to the Sertoli cells as seen during the period of their development.

The desquamated cells present the widest variety of forms. Figs. 5 and 6 give a better idea of this than can be gained from detailed descriptions. The size of many of these cells is surprising, and the presence of more than one pycnotic nucleus in many of them suggests an incomplete separation of the protoplasm, or else the assumption of a globular form by multinucleate portions of the epithelial wall. The nuclei correspond in size and shape to spermatids in most instances. Giant cells which frequently occur here will be described more fully later. Although larger nuclei may represent incompletely divided ones, various forms of caryorrhexis are seen, some involving nuclei larger than any still attached to the wall. The fact that these processes begin while the cells form a part of the epithelium is shown in the drawings. The protoplasm of all these cells is practically homogeneous, contains occasional vacuoles, and has an increased affinity for eosin. Hyaline globules taking a fainter red stain make up a small part of this mass, and their origin in the wall is shown in Fig. 5. They occur normally to about the same extent.

The epithelial layer as a whole is occasionally loosened from the basement membrane, but in the absence of definite evidence to the contrary this condition is regarded as an artifact. The focal nature of the process is strongly suggested in many sections by the varying intensity of the injury to the tubules, thus corresponding to the action of infections upon other organs.

Slight changes in the basement membrane may be due in some cases to apparent thickening which follows an attempt to conform to a tubule of diminished size. More extensive alteration than this is observed in some instances.

The interstitial tissue is subject to considerable variation, some sections showing no increase while others are fibrosed quite irregularly. Occasional areas of round cell infiltration are to be seen about the smaller blood vessels.

The cells of Leydig are generally unchanged, but in some instances they appear to be slightly increased. Such a change might be expected in association with destruction of tubules, but no mitotic figures are to be seen and constructive tissue activity in the presence of a fatal infection is most unlikely. If present the increase must be attributed to previous activity.

In the older, more advanced stages the hyaline membrane continues to increase in thickness with a corresponding decrease in the epithelial cells. A few fibroblasts appear on the inner edge of the membrane and persist until all the Sertoli cells have disappeared. The tubule is now less than half its original size, its lumen is obliterated, and its walls are represented by wavy acellular hyaline material. The size is further diminished and the hyaline material gradually merges with the invading fibroblasts until the tubule is completely lost in the irregular bands of connective tissue that traverse the testes and characterize the condition called fibrous orchitis. The last traces of the tubules are indicated by the suggestive arrangement of the fibroblast nuclei in the periphery, but eventually these too become indistinguishable.

There is no reason to think that any particular part of the process is peculiar to pneumonia or definitely assignable to it. On the contrary, there is every reason to believe that the testicular change found at the death of a pneumonia patient depends chiefly upon the time that the disease has been operative as measured by the number of days in the hospital. Other factors doubtless influence the picture, such as the intensity of the infection, the predominance of certain organisms, etc., but unquestionably the most potent factor is the length of the illness.

In the tables the cases have been so grouped that Table I includes the cases of primary pneumonia that had no antecedent infectious disease, while Tables II and III describe those following measles and epidemic influenza.

Cases of Pneumonia in Which Streptococcus hæmolyticus Occurs.

Primary Infections (Table I, A).—There are eleven cases in this series, in which the hemolytic streptococcus alone was found in eight, and associated with other organisms in three. The lobar type of

TABLE I.

Autopsy No.	Length of time in hospital.	Associated organisms.	Principal anatomical diagnosis.	Sertoli cells.	Spermatogonia.	Spermatocytes.	Spermatids.	Spermatozoa.	Desquamated cells.	Giant cells.	Basement membrane.	Interstitial tissue.	Edema.	Older lesions.
A. *Streptococcus hæmolyticus*, occurring in a primary pneumonia, not following an epidemic disease.														
1. Streptococcus alone.														
	days													
53	33		Pulmonary abscesses; empyema.	Norm.	Norm.	Few.	—	—	—	—	Sl. thick.	Incr.	+	—
56	28		Lobular.	Decr.	Decr.	"	—	—	—	—	Thick; hyaline.	" *	+	+
57	36		Atelectasis; empyema, pericarditis.	Norm.	Norm.	"	—	—	—	—	Thick; hyaline.	" *	—	+
58	55		IBP; lobular; empyema.	"	"	"	—	—	—	—	Thick.	"	—	—
171	32		" empyema; acute nephritis.	Decr.	Decr.	—	—	—	—	—	Sl. thick.	Sl. incr.	—	—
174	32		IBP; empyema.	Norm.	Norm.	—	—	—	Rare.	—	" "	Norm.	+	—
192	8	St.	Lobular; ulcerative laryngitis.	"	"	Decr.	Decr.	Few.	Many.	Few.	Norm.	"	+	—
193	14	SNh.	IBP.	"	"	"	Few.	Rare.	Few.	Rare.	Sl. hyaline.	Sl. incr.	—	—
2. With *B. influenzæ*.														
191	31		IBP; empyema.	Norm.	Norm.	Few.	—	—	Few.	Rare.	Sl. thick; hyaline.	Incr.	—	+
3. With pneumococcus.														
185	3(?)		Lobar; bronchitis; meningitis; pericarditis.	Norm.	Norm.	Decr.	—	—	Few.	—	Sl. thick.	Sl. incr.	—	—

4. With *B. influenzæ* and pneumococcus.

186	12		Lobar; empyema; pericarditis.	Norm.	Norm.	Decr.	Few.	—	Some.	Few.	Sl. thick.	Incr.	—	+

B. *B. influenzæ*, occurring in a primary pneumonia, not following an epidemic disease.

1. *B. influenzæ* alone. No cases.

2. With *Streptococcus hæmolyticus*. Identical with A 2 above.

3. With pneumococcus.

51	18		Lobar; peritonitis.	Decr.	Decr.	Few.	Few.	—	Some.	—	Norm.	Norm.	++	—
188	8		" acute nephritis.	"	"	"	—	—	Few.	Rare.	Sl. thick.	Much incr.	—	+

4. With *Streptococcus hæmolyticus* and pneumococcus. Identical with A 4 above.

C. Pneumococcus, occurring in a primary pneumonia, not following an epidemic disease.

1. Pneumococcus alone.

19	5		Lobar; empyema; pericarditis.	Norm.	Norm.	Decr.	Decr.	Decr.	Few.	—	Sl. hyaline.	Incr.*	—	?
25	7		"	"	"	"	"	"	"	—	" "	Sl. incr.	—	—

2. With *Streptococcus hæmolyticus*. Identical with A 3 above.

3. With *B. influenzæ*. Identical with B 3 above.

4. With *Streptococcus hæmolyticus* and *B. influenzæ*. Identical with A 4 and B 4 above.

IBP has been used for interstitial bronchopneumonia, CLP for confluent lobular pneumonia, and BP for bronchopneumonia. − indicates absence and + presence, while an asterisk denotes the occurrence of small areas of round cell infiltration in the interstitial tissue. In explanation of the meaning of "some" in this connection, it might be said that this is arbitrarily taken as the equivalent of the number of the spermatogonia in any given tubule and hence the terms "many," "few," and "rare" have correlated significance. Norm. indicates normal; decr., decreased; incr., increased; sl., slight or slightly. St. means staphylococcus; StA, *Staphylococcus aureus;* BMC, *B. mucosus capsulatus;* TB, *B. tuberculosis;* Gas B, gas bacillus; SNh, non-hemolytic streptococcus.

lung involvement was found in two cases and five had interstitial bronchopneumonia, while nearly all had empyema or some complication of an infectious nature.

The men were not debilitated by antecedent disease and hence the course of the pneumonia was longer. If the pneumonia is more important in the causation of the testicular changes than an antecedent disease, then these cases of primary infections should show more extensive lesions. It is fair to assume that a septicemia of severe grade and perhaps long duration has been present in all the cases, and in this event the organisms have had abundant opportunity to affect the different parts of the body in an injurious way. That this was true is evident from the extent to which the testes were injured. The Sertoli cells, which have been found to be the most resistant of all the cells in other forms of testicular lesions, suffered evident injury in two cases, and the derived cells were absent in all but three. These instances of extensive and fundamental injury were well distributed over the four subgroups divided according to the presence and kind of associated organisms, and the impression is therefore gained that the presence of other organisms is of no special significance as determining at least this part of the pathology of primary pneumonia.

The spermatogonia were injured and decreased in the same cases in which the Sertoli cells suffered. Spermatozoa were absent in every case but two, and in these they were represented by mere traces. In no instance were they normal in structure, but represented the last stages before complete removal. In one of these cases, No. 193, the section of the seminal vesicle shows that these bodies had been active there until shortly before death.

The microscopic picture presented by the sections was most varied. Nearly all the types of lesions were represented by at least one case, and three was the largest number of instances in which a single type occurred. Acute lesions were found in eight cases, and the more chronic or older lesions occurred in four. This is to be correlated with the fact that very old lesions occur in four instances, that the interstitial tissue is definitely increased in nine, and that two of these have areas of round cell infiltration.

It is perhaps significant to note that ten out of the eleven had definite changes in the thickness and character of the basement membrane.

This has been found to indicate quite clearly the amount of fundamental injury done and to be one of the essential features of the lesions associated with complete atrophy, hyalinosis, and gradual obliteration of the tubules. It is reasonable, therefore, to assume that its initial stages are of some significance, inasmuch as there is no reason to suppose that hyaline changes once begun can be removed with complete restitution of functional integrity.

Streptococcus hæmolyticus Infections Following Measles (*Table II, A*).—The length of hospital treatment as compared with that in the previous table does not seem to be greater, in spite of the known antecedent disease. In the series of eighteen cases only three had lobar involvement, in two of which the pneumococcus was associated; six had various forms of lobular consolidation, and twelve had the form known as interstitial bronchopneumonia. The last finding is the more surprising in view of the conclusion based upon histological grounds that this represents a tissue-resistant type of disease and the fact that measles has the ability to lower greatly the resistance of the body. This may be the explanation of the relatively shorter period of illness, in that the terminal lung infection caused the death of the patient much more quickly when the body resistance had been previously reduced. If this explanation is true, then the shorter exposure to the effects of the streptococcus would have resulted in less extensive changes in the tubule epithelium. This may be borne out by the fact that only one section showed evidence of injury to the Sertoli cells and two to the spermatogonia. The acute nature of the injury operating over a short period of time is expressed in the finding that all the sections but two contained varying numbers of loose cells in the lumen of the tubules and that there had not been time enough after the injury for them to be completely removed.

On the other hand, the action of the measles in inhibiting the production of spermatozoa is indicated by the fact that they were absent in every section except one. The interpretation of the series is that an influence inhibiting spermatogenesis to a moderate degree had been operative during a sufficient period for spermatozoa already formed to be removed, and that a more acute injurious process had supervened, loosening many of the remaining cells and causing death before they too could be removed. The lesions were diversified, the

TABLE II.

Autopsy No.	Length of time in hospital.	Associated organisms.	Principal anatomical diagnosis.	Sertoli cells.	Spermatogonia.	Spermatocytes.	Spermatids.	Spermatozoa.	Desquamated cells.	Giant cells.	Basement membrane.	Interstitial tissue.	Edema.	Older lesions.
A. *Streptococcus hæmolyticus*, occurring in a pneumonia following measles.														
1. Streptococcus alone.														
	days													
206	6(?)		Lobular; empyema.	Norm.	Norm.	Decr.	Rare.	Many.	Many.	—	Norm.	Norm.	—	—
34	14		IBP; empyema.	"	"	Few.	Few.	—	Few.	—	Sl. hyaline.	Incr.*	+	+
41	17		" "	"	"	Decr.	"	—	Some.	Rare.	" thick.	"	—	+
50	36		" lobar; empyema.	"	"	"	"	—	"	"	" hyaline.	Sl. incr.	+	—
55	25		Lobular; septicemia; empyema.	Decr.	Few.	—	—	—	—	—	" thick.	Norm.	+	+
176	45		IBP; empyema; pericarditis; abscesses.	Norm.	Norm.	Decr.	—	—	Few.	—	Norm.	"	+	—
178	17	StA.	IBP; ulcerative laryngitis.	"	"	Few.	—	—	Some.	Rare.	Sl. thick.	Sl. incr.	—	+
184	34		" " "	"	"	Decr.	Few.	—	Many.	Few.	" "	Incr.	—	—
194	13		" lobular; empyema; ulcerative laryngitis.	"	"	"	—	—	Some.	"	" "	Sl. incr.	—	—
195			Lobular; empyema.	"	"	"	Few.	—	Many.	"	" "	" "	—	—
204	11		IBP, with abscesses; empyema; pericarditis.	"	"	Few.	—	—	Few.	—	" "	" "	+	—
2. With *B. influenzæ*.														
32	7(?)		IBP.	Norm.	Norm.	Few.	—	—	Few.	—	Norm.	Sl. incr.*	—	+
37	16		" empyema.	"	"	Norm.	Decr.	—	Some.	Few.	"	Norm.	+	—
202	21		BP; "	"	Decr.	—	—	—	Few.	—	Sl. thick.	Incr.	—	+
205	25		IBP; lobular; empyema; otitis media.	"	Norm.	Decr.	Few.	—	"	Rare.	" "	"	—	?

3. With pneumococcus.															
31 46	25 5		IBP; lobar; empyema. Lobar; empyema; pericarditis.	Norm. "	Norm. "	Few. Decr.	– Few.	– –	Few. Some.	– Rare.	Sl. hyaline. " thick.	Sl. incr.* Incr.	+ +	+ –
4. With *B. influenzæ* and pneumococcus.															
33	35		Lobar; empyema.	Norm.	Norm.	Few.	–	–	–	–	Sl. thick.	Sl. incr.	+	+
B. *B. influenzæ*, occurring in a pneumonia following measles.															
1. *B. influenzæ* alone. No cases.															
2. With *Streptococcus hæmolyticus*. Identical with A 2 above.															
3. With pneumococcus. No cases.															
4. With *Streptococcus hæmolyticus* and pneumococcus. Identical with A 4 above.															
C. Pneumococcus, occurring in a pneumonia following measles.															
1. Pneumococcus alone. No cases.															
2. With *Streptococcus hæmolyticus*. Identical with A 3 above.															
3. With *B. influenzæ*. No cases.															
4. With *Streptococcus hæmolyticus* and *B. influenzæ*. Identical with A 4 and B 4 above.															

TABLE III.

Autopsy No.	Length of time in hospital.	Associated organisms.	Principal anatomical diagnosis.	Sertoli cells.	Spermatogonia.	Spermatocytes.	Spermatids.	Spermatozoa.	Desquamated cells.	Giant cells.	Basement membrane.	Interstitial tissue.	Edema.	Older lesions.
A. *Streptococcus hæmolyticus*, occurring in a pneumonia following epidemic influenza.														
1. Streptococcus alone.														
219	*days* 10		CLP; pleurisy.	Norm.	Norm.	Decr.	Few.	Few.	Some.	Few.	Norm.	Sl. incr.	–	–
2. With *B. influenzæ*.														
89	24		IBP; pericarditis.	Norm.	Norm.	Few.	Few.	–	Few.	–	Sl. thick.	Incr.	–	+
3. With pneumococcus.														
226		St.	CLP; pleurisy.	Norm.	Norm.	Norm.	Decr.	Few.	Some.	Rare.	Norm.	Sl. incr.	–	?
4. With *B. influenzæ* and pneumococcus.														
83	11	St.	CLP; pleurisy.	Norm.	Norm.	Decr.	Decr.	Some.	Few.	Rare.	Norm.	Norm.	–	–
88	15	StA.	IBP; " myocarditis.	"	"	Few.	Few.	–	"	–	"	"	–	–
B. *B. influenzæ*, occurring in a pneumonia following epidemic influenza.														
1. *B. influenzæ* alone.														
81	19		IBP; pleurisy.	Norm.	Norm.	Few.	Few.	–	Some.	Rare.	Norm.	Incr.*	–	–
86	21		" " endocarditis.	"	"	"	–	–	Few.	–	Sl. thick.	Sl. incr.*	–	?
211	9	StA.	CLP.	"	"	Decr.	Decr.	Some.	Some.	Few.	" hyaline.	Norm.	+	–
2. With *Streptococcus hæmolyticus*. Identical with A 2 above.														
3. With pneumococcus.														
80	15	St.	CLP; pulmonary embolism.	Norm.	Norm.	Decr.	Decr.	Decr.	Few.	Rare.	Sl. thick.	Sl. incr.	–	–
82	7	"	" pleurisy; ulcerative laryngitis.	"	"	"	"	Some.	Some.	Few.	" hyaline.	" "	–	–

84	11	St.	CLP; ulcerative laryngitis; endocarditis.	Norm.	Norm.	Few.	—	—	Some.	Rare.	Norm.	Sl. incr.	+	+
85	10	BMC.	CLP; jaundice.	"	"	"	—	—	Few.	"	Sl. thick; hyaline.	Incr.	—	+
87	17	"	IBP, with pulmonary abscesses; empyema; endocarditis.	"	"	—	—	—	—	—	Thick; hyaline.	"	—	+++
90	23	TB.	Lobular; tuberculous cavities.	"	"	Few.	—	—	Few.	Rare.	Sl. thick; hyaline.	" *	—	+
214	13		CLP.	"	"	Decr.	Few.	Few.	Many.	Some.	Sl. hyaline.	"	—	+
215	10	St.	" pleurisy; ulcerative laryngitis.	"	"	"	"	"	Few.	Rare.	Norm.	Sl. incr.	—	—
216	15		CLP; pleurisy.	"	"	"	"	Rare.	"	"	Sl. thick.	Incr.	—	+

4. With *Streptococcus hæmolyticus* and pneumococcus. Identical with A 4 above.

C. Pneumococcus, occurring in a pneumonia following epidemic influenza.

1. Pneumococcus alone.

212	19		CLP; pleurisy.	Norm.	Norm.	Decr.	Some.	Some.	Few.	Rare.	Norm.	Norm.	—	+
217	23		" " emphysema (Gas B).	"	"	"	Few.	—	Some.	Few.	Sl. hyaline.	Incr.	—	+
220	15	TB.	CLP; pleurisy; tubercles in spleen.	"	"	"	"	Few.	Many.	"	" thick.	"	—	+
221	11		CLP; pleurisy.	"	"	"	"	"	Some.	"	Norm.	"	—	?
222			" "	"	"	Norm.	Norm.	Some.	"	"	Sl. hyaline.	Sl. incr.	—	—
223	9		" "	"	"	Decr.	Decr.	Decr.	"	Rare.	" "	" "	—	—
224	9		" "	"	"	"	"	"	Few.	"	Norm.	Incr.	—	—
227			" "	"	"	"	"	Many.	Some.	Few.	"	Sl. incr.	—	?
228	12		" "	"	"	Few.	—	—	Few.	Rare.	Sl. thick.	" "	—	—
229	10		" " mitral stenosis.	"	"	Decr.	Decr.	Few.	"	"	" hyaline.	" "	—	—

2. With *Streptococcus hæmolyticus*. Identical with A 3 above.

3. With *B. influenzæ*. Identical with B 3 above.

4. With *Streptococcus hæmolyticus* and *B. influenzæ*. Identical with A 4 and B 4 above.

type in Fig. 6 being found in six sections. The chronic types of lesions appeared in seven cases, while evidence of acute change was seen in sixteen. Very old lesions were seen in eight slides, and those of a doubtful character in still another. Interstitial tissue increase affected all but four cases. Moreover, the basement membrane was thickened in all except four. Round cell infiltration appeared in three cases and the so called edema in nine.

No obvious difference can be made out between the cases in which the streptococcus occurred alone and those in which it was associated with one or more pathogenic forms.

Streptococcus hæmolyticus Infections Following Epidemic Influenza (*Table III, A*).—The type of lung lesion is still different in these cases, there being no lobar consolidations, and only two cases of interstitial bronchopneumonia. The remaining three were diagnosed confluent bronchopneumonia, a type of lung lesion supposed to be low in the scale of resistance. Pleurisy accompanied nearly all these cases and empyema was completely absent. The pneumonia was the terminal condition in every instance, and nothing of a more prolonging nature occurred as a complication or sequel. The period of illness incident to the influenza was as usual short, and the pneumonia following was also of short duration. As a result the stay in the hospital was from 10 to 24 days, averaging a much shorter time than in the two preceding series. Subdivision of these cases gives a definitely longer period of illness for those having interstitial pneumonia than those with the confluent type.

The microscopic findings correspond closely with this picture. The Sertoli cells and spermatogonia have been apparently uninjured. The spermatocytes and spermatids have been much reduced in all cases but not completely removed in any, and the spermatozoa are still present in three out of five. Loosened cells occur in all, and the basement membrane has been thickened only in the case that lived the longest. The whole picture indicates clearly that the preceding influenza had little effect in the inhibition of spermatogenesis, possibly because of the short time in which it could manifest itself and because the pneumonia that followed was so fulminant in character that the patient died before the cells of the tubules were more than loosened from their attachments.

Cases of Pneumonia in Which Bacillus influenzæ Occurs.

Primary Infections (*Table I, B.*)—These cases developed during the measles epidemic, but so far as could be learned they had not had that disease. There were no instances in which the organism occurred alone, and those in which the streptococcus was found have been previously described. In three of the four cases the lobar type of lung involvement was found, and one had interstitial bronchopneumonia. The two cases in which the pneumococcus was also found are apparently more severely injured than that associated with the streptococcus; however, the case affected by all three organisms manifested the least injury. There was a definite lack of uniformity about the picture presented by this group, suggesting that although it belonged to a supposedly primary infection, the associated organisms were perhaps more potent than the one in common. This fact suggests that *Bacillus influenzæ* is not of importance as a cause of testicular change.

Bacillus influenzæ Infections Following Measles (*Table II, B*).—There were no cases in which *Bacillus influenzæ* was found alone or associated with the pneumococcus. The entire five cases were associated with the streptococcus alone or with the latter and the pneumococcus. The conclusion is therefore readily drawn that the streptococcus is the dominant organism, and a discussion of its effects here would be unwarranted.

Bacillus influenzæ Following Epidemic Influenza (*Table III, B*).—Three cases are reported in which this organism occurred alone, one with the streptococcus, nine with the pneumococcus, and two with both. The cases with the streptococcus or without other organisms were usually of the interstitial bronchial type, while those in which the pneumococcus was found were largely of the confluent lobular variety. One case had also small tuberculous cavities. Prolonging complications were absent and the longest period of illness was 24 days. Possibly the shortness of the time has influenced the relative uniformity of the picture inasmuch as the various groups have no observable differences. The spermatogonia and Sertoli cells were not obviously affected in any case. The interstitial tissue of the sections was increased in two. Perhaps this was just a coincidence, as it is hard to understand how an acute infection could have produced such a result. The presence of old lesions accompanied this increase in most instances.

Cases of Pneumonia in Which the Pneumococcus Occurs.

The cases of pneumonia as they occurred among the civil population under pre-war conditions have been considered to be caused chiefly by the pneumococcus. Occasional mention of *Streptococcus hæmolyticus*, and more rarely of the influenza bacillus, has been made, but on bacteriological and serological grounds the pneumococcus has been regarded as the chief etiological factor. Doubtless the attention that has been given the other forms during the present epidemics will serve to emphasize their importance and lead to their recognition in the future in a larger percentage of cases. It remains to be seen how long they will retain their activity in the causation of pneumonia after camp concentration has ceased, and a satisfactory determination of this point will enable one to review these cases and to pass more mature judgment than is now possible. The impression seems to be that the original importance of the pneumococcus in the production of pneumonia was, at least during the measles epidemic, superseded by *Bacillus influenzæ* or *Streptococcus hæmolyticus* or both these organisms. The cases described by Cordes were doubtless similar to other pre-war cases and were not even comparable from an etiological standpoint with any of the present series of cases. The latter, it must be remembered, were probably caused by the same organisms that produced the pneumonia directly following the epidemic diseases, and were perhaps more virulent than usual, an idea strongly supported by the extraordinary incidence of pneumonia at that time.

Primary Infections (*Table I, C*).—Six cases are collected and in all the lobar type of consolidation was found. Several had complications such as empyema, peritonitis, and pericarditis, but most of them terminated after short periods of illness. Two cases were found at the beginning of the measles epidemic, in which the pneumococcus occurred alone, all the later ones being complicated by the presence of the streptococcus and the influenza bacillus.

The type of pneumococcus was not uniform, all the types being represented in about the same proportions.

The pathological picture of the cases with only the pneumococcus present was relatively simple and uniform. Spermatogenesis was not completely stopped, as evidenced by the presence of at least a few

spermatozoa and only a moderate degree of reduction in the derived cells. The basement membrane was only slightly hyalinized and the interstitial tissue was slightly increased. All but one of these six cases had thickened basement membranes and in nearly all the interstitial tissue was increased. The one without changes in the basement membrane or increase in the interstitial tissue had a pronounced separation of the tubules which has been described as edema.

Pneumococcus Infections Following Measles (*Table II, C*).—There were no cases in which this occurred alone or in conjunction with *Bacillus influenzæ*. The three cases enumerated were all associated with the streptococcus, alone or in conjunction with *Bacillus influenzæ*. The importance of the streptococcus in this group of postmeasles cases is emphasized by the relative unimportance of the pneumococcus and the influenza bacillus. The details of the picture have been sufficiently discussed above.

Pneumococcus Infections Following Epidemic Influenza (*Table III, C*).—The influence of the pneumococcus seems to be relatively greater in this series than it was in those preceding. There were ten cases in which this was the only organism found, one in which it was associated with the streptococcus, nine with *Bacillus influenzæ*, and two with both. Those with a single infection were uniform in the general disease picture, having simply confluent lobular pneumonia and pleurisy. In the other groups in which other organisms perhaps played a part the same general features were repeated with little variation.

The Sertoli cells and spermatogonia were unaffected, and only moderate degrees of injury to the derived cells were seen. Spermatozoa were still present in all but seven cases, and spermatids persisted in all but five. The types of lesion in almost every instance corresponded to those in Fig. 5.

DISCUSSION.

Presumably there exists a period following the onset of an infection, if this is rather sudden and acute, in which the testes show little evidence of injury. Somewhat analogous conditions are found in early death from mercuric chloride poisoning in which the kidneys have had little time to give visible evidence of the chemical injury done. Such an overwhelming bacterial intoxication is not seen in pneumonia, and

hence this prereactionary stage is not represented in the series. From febrile changes in other organs it would seem that these morphological alterations should begin to appear rather early and hence would be well under way within the period of an ordinary pneumonia.

The separation of the cells in the epithelial wall somewhere central to the row of Sertoli cell nuclei, which is an occasional finding, causes a loosening of the spermatids and of some of the spermatocytes. If the Sertoli cells receive nourishment from the cells of Leydig, as is commonly believed, it is reasonable to suppose that protoplasmic changes might early affect the nutrition of the derived cells. Hanes (7) believes that one function of the Sertoli cells is to supply fat to the developing sperm cells, and hence it may be assumed that any interruption of this process would lead to fragmentation of the protoplasm with loosening of these cells.

If, on the other hand, the toxic action is slower and more continuous, the same result would appear in a more insidious and gradual manner. This is really more nearly according to facts, in that a fragility exists which leads to breaks in the continuity.

It is conceivable that mitotic activity of sperm cells would be inhibited in much the same way that other functions of epithelium are reduced in fevers; for example, the secretion of the sebaceous glands. These would naturally resume activity if recovery from the initial disease resulted, but in fatal cases would show no tendency toward restoration.

However, inhibition of spermatogenesis is not always a pathological process. Investigations of Hansemann (8) on hibernating and waking marmots show that this change occurs normally and that restoration consists of the formation of a new supply of derived cells by multiplication of the spermatogonia that have been for a time quiescent. Grandis (9) found that spermatogenesis ceased in fasting doves after a few days; the spermatozoa already formed were speedily removed and most of the cells in the tubules died. The detritus appeared to be diverted for the nourishment of the bird but the spermatogonia persisted and formed new elements after the hunger was relieved. There is no reason, therefore, for regarding the mere death and removal of the derived cells as unusual, when we consider the metabolic drain imposed upon the system by an acute and prostrating fever. Injury to

the spermatogonia and perhaps changes in the supporting framework are, however, matters of special concern. The method by which the damage is accomplished and the variations in the appearance of the products of degeneration are worthy of study as possible indices of the cause of the injury and a measure of its power.

Theoretically if the infection is responsible for the testicular injury, the greatest degree of alteration would be shown in the lists of cases in which that agent was the common factor. Variations might occur in the severity in proportion to the virulence of the associated organisms or prominence of other pathological conditions. Uniformity of results in any one table, then, would strongly suggest the paramount potency of the one or two common factors.

However, to differentiate the true lesions of pneumonia from the changes in the testes properly attributable to autolysis and postmortem alteration is not always easy. Most of the autopsies in this series were done shortly after death in order to secure cultures of organisms as free as possible from contaminations. It is therefore unlikely that more than 3 or 4 hours elapsed before the tissues were placed in fixing fluid.

The suggestion has been made that the separation of the epithelium from the basement membrane and the consolidation of the former in the interior with loss of lumen are not a technical fault, but evidence of a serious injury that leads to atrophy. Such distortions are common in various poorly fixed or greatly shrunken tissues and hence such an interpretation here would not be unwarranted. On the other hand, in sections in which there has been little or no cellular decrease and those from accident cases in which infection and other morbid influences had little time to act, this condition was absent. Very rarely are healthy spermatozoa and skrunken tubules found in the same section.

A condition resembling edema has been portrayed in Figs. 7 and 8, and has been described before. Councilman, Magrath, and Brinckerhoff (10) mention an edema of the interstitial tissue in smallpox in relation to similar epithelial changes in the testes. In pneumonia an acute alteration in the tubules, often indicated by the coexistence of a definite shrinking of the epithelial masses and a rarefaction of the interstitial tissue, has been described by Weichselbaum and Kyrle

as edema. This coincidence exaggerates the epithelial change and is quite striking. In these spaces, however, granules of coagulated protein are usually lacking.

The interstitial tissue is also subject to increase in density. This may be a diffuse process or may take the form of irregular bands of connective tissue, more or less associated with hyalinized tubules. The blood vessels are thickened in some instances. Weichselbaum and Kyrle consider that the desquamation of cells is a part of the pathological picture, whereas Cordes believes it to be a postmortem phenomenon. This series of cases points definitely to the correctness of the former statement. It seems clear that the number of desquamated cells progressively decreases after the initial injury, and that, other things being equal, the number of loosened or injured cells remaining is an index of the length of this period.

The presence of giant cells is one of the structural features that call attention to the abnormality of the process. They have been described before in a variety of testicular lesions, but until recently no explanation of their origin was offered. Weller (11) apparently correctly considers them to be the result of attempts at cell division in which the separation of the protoplasm is delayed. The nuclei are usually peripherally arranged and may surround a vacuole. The size of the nuclei is variable, some corresponding to those of spermatids and others being somewhat smaller. The nuclei number from two to six or more and correspond structurally with the spermatids in which pycnosis has taken place.

The point of formation is definitely shown in Fig. 5, in which a fully developed giant cell is still attached in the zone of the spermatids. It is hardly likely that further development takes place after desquamation. No mitotic figures have been seen in them and no evidence of phagocytosis. The protoplasm stains uniformly but heavily with eosin, and the cell aggregate has assumed a circular form, doubtless as a result of cohesive force. The number of these giant cells is variable. They are most numerous where the amount of desquamated material is greatest, but rapidly diminish as the other cells disappear, being among the first to go. They are more abundant in the simplest cases of postinfluenzal pneumonia where the number of desquamated cells is greatest.

SUMMARY.

The testicular changes in pneumonia are without clinical manifestations, are non-specific, focal in character, independent of the infecting organisms or the antecedent disease, and vary in severity directly with the total length of the illness. The process is a continuous one, divisible into stages in which the following features are recognizable: (1) cessation of spermatogenesis; (2) degeneration of preformed spermatocytes, spermatids, and spermatozoa; (3) desquamation of altered cells and fragments of the same; (4) formation of giant cells in the tubule walls with subsequent liberation into the lumen; (5) disappearance of all desquamated cells and all those derived from the spermatogonia by mitosis; (6) in some instances thickening of the hyaline layer of the basement membrane.

Older lesions are frequently found which continue the structural alteration of the tubules by hyalinosis and destruction of cells until they ultimately disappear. These lesions are not believed to be connected with the present illness.

Edema may represent the acute injury in another form, and round cell infiltration suggests that possibly other factors than toxins may have a part in the tissue alterations.

In the absence of definite evidence to the contrary, the cause is assumed to be circulating toxins, as Wolbach (12) claims for influenzal cases.

The hemolytic streptococcus produced more extensive changes, both epithelial and interstitial, in primary pneumonia occurring during the measles epidemic than when pneumonia followed as a secondary infection; in the latter cases the pulmonary complications covered a relatively shorter period. Measles and epidemic influenza had little apparent effect upon the testes, except that the former caused mild inhibition of spermatogenesis; evidence regarding the latter is inconclusive.

The Pfeiffer bacillus was always associated with other organisms, in primary infections and in those following measles. It occurred alone in a few cases after epidemic influenza, but the testicular lesion was not distinctive.

The pneumococcus when alone in primary infections or after an epidemic disease produced a uniformly mild picture which was not intensified when associated with the influenza bacillus.

Giant cells were much more frequent after influenzal pneumonia regardless of its cause and were associated with large numbers of other desquamated cells. They are formed in the walls of tubules by futile mitotic effort and incomplete protoplasmic separation, the abnormality of the process being further suggested by the early severing of cytoplasmic attachments and rapid desquamation.

The series is unique in its uniformity, in the care exercised in the bacteriological examinations, and in the relative freedom from complicating factors.

The author is under deep obligation to Professor W. G. MacCallum for material and cordial cooperation.

BIBLIOGRAPHY.

1. Osler, W., and McCrae, T., Modern medicine, Philadelphia and New York, 2nd edition, 1913, i.
2. Cordes, H., *Virchows Arch. path. Anat.*, 1898, cli, 402.
3. MacCallum, W. G., *J. Am. Med. Assn.*, 1919, lxxii, 193.
4. MacCallum, W. G., The pathology of the pneumonia in the United States Army Camps during the winter of 1917–18, Monograph of The Rockefeller Institute for Medical Research, No. 10, New York, 1919.
5. MacCallum, W. G., *J. Am. Med. Assn.*, 1919, lxxii, 720.
6. Weichselbaum, A., and Kyrle, J., *Sitzungsb. k. Akad. Wissensch. med.-naturw. Kl.*, 1912, cxxi, 51.
7. Hanes, F. M., *J. Exp. Med.*, 1911, xiii, 338.
8. Hansemann, D., *Virchows Arch. path. Anat.*, 1895, cxlii, 538.
9. Grandis, V., *Arch. ital. biol.*, 1889, xii, 215.
10. Councilman, W. T., Magrath, G. B., and Brinckerhoff, W. R., *J. Med. Research*, 1904, xi, 12.
11. Weller, C. V., *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 14.
12. Wolbach, S. B., *Bull. Johns Hopkins Hosp.*, 1919, xxx, 104.

EXPLANATION OF PLATES.

The illustrations were drawn with a camera lucida at a magnification of 500 diameters. The cells are therefore comparable in size, but for convenience the smallest tubule that would show the lesion was selected.[2]

[2] The helpful suggestions of Professor Max Brödel are greatly appreciated.

PLATE 45.

FIG. 1. Case 165. A quiescent stage in which only spermatogonia and Sertoli cells remain. The protoplasm of the latter is merely a fine network, some of the holes in which represent the places from which the derived cells have been removed.

FIG. 2. Case 216. Further reduction with persistence of Sertoli cells only. Shrinkage of the wall probably due to faulty technique.

PLATE 46.

FIG. 3. Case 216. A special form of protoplasmic change in which large deep red granules obscure all traces of cellular structure. Note the relation to adjacent protoplasm suggesting infiltration or expansion and lack of continuity with neighboring cells.

PLATE 47.

FIG. 4. Case 216. A more advanced stage of the process shown in Fig. 3. The nuclei are not materially affected and apparently belong to Sertoli cells. An unaffected tubule is shown in the upper left corner and the mass of connective tissue on the right is part of a heavy band containing remnants of destroyed tubules, suggesting that this process is definitely destructive.

PLATE 48.

FIG. 5. Case 227. A common picture in postinfluenzal cases. Large numbers of desquamated cells including giant cells, hyaline bodies, and spermatozoa. Cells with deep red homogeneous protoplasm are breaking away from the free margin and one giant cell is still embedded in the wall.

PLATE 49.

FIG. 6. Case 90. Fewer desquamated cells and greater reduction in the derived cells. The Sertoli cells are quite definite as the second row, and the spermatogonia next to the wall are moderately well preserved. The basement membrane is slightly thickened and thrown into small folds.

PLATE 50.

FIG. 7. Case 51. Great edema of the interstitial tissue with collapse of tubule walls upon the decreased cellular mass. Acute degeneration of all the cells including spermatogonia and Sertoli cells.

FIG. 8. Case 53. Collapse of tubule walls and adjacent tissue upon a tubule in a resting stage, similar to that in Fig. 1. Edema of the interstitial tissue.

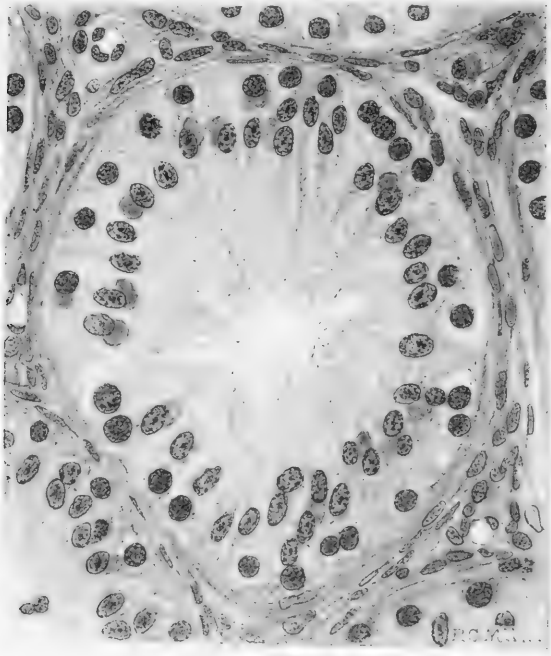


FIG. 1.

FIG. 2.

(Mills: Changes in testes in pneumonia.)

FIG. 3.

(Mills: Changes in testes in pneumonia.)

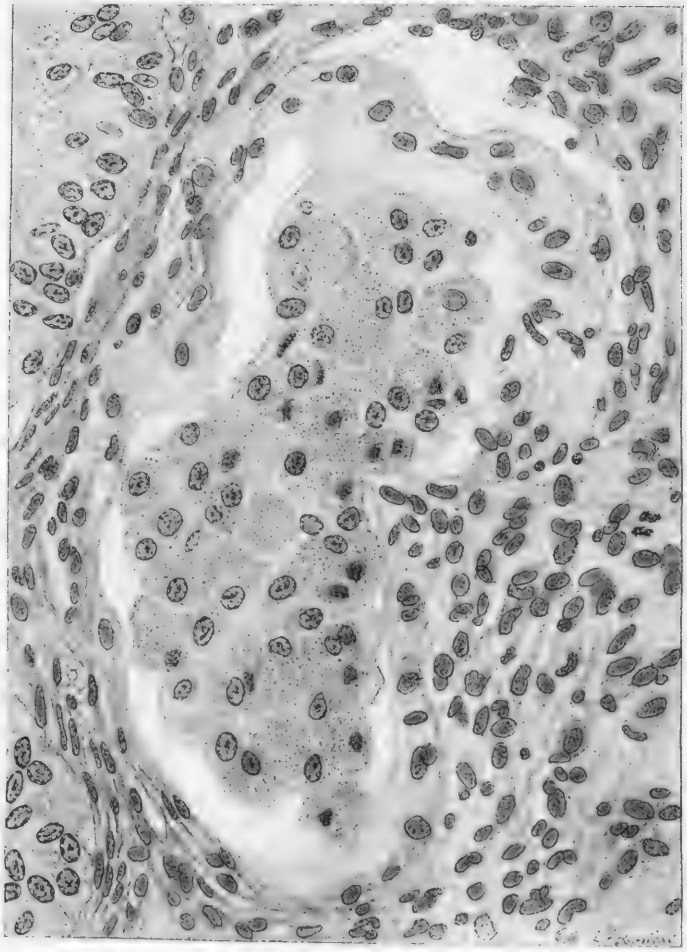


FIG. 4.

(Mills: Changes in testes in pneumonia.)

FIG. 5.

(Mills: Changes in testes in pneumonia.)

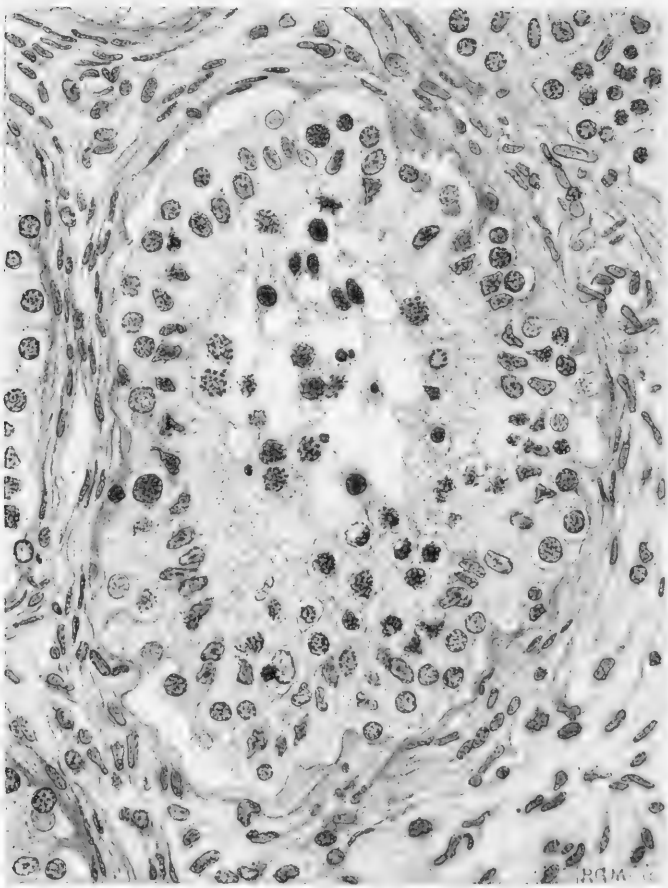


FIG. 6.

(Mills: Changes in testes in pneumonia.)

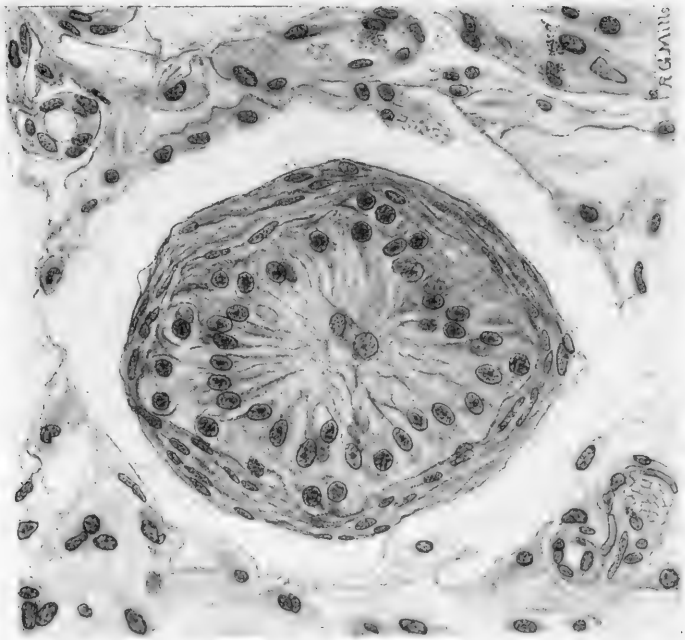


FIG. 8.

FIG. 7.

(Mills: Changes in testes in pneumonia.)

The Journal of General Physiology

Edited by

JACQUES LOEB, New York, N. Y.

W. J. V. OSTERHOUT, Cambridge, Mass.

The Journal of General Physiology is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter.

The Journal of General Physiology is issued bimonthly, one volume of about 600 pages appearing in a year. Contributions should be sent to the editors of *The Journal of General Physiology*, The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y., or 60 Buckingham Street, Cambridge, Mass. The papers should be limited preferably to twenty printed pages, not counting the space occupied by illustrations. Authors receive 100 reprints of their papers free of charge; additional copies may be obtained at cost.

The subscription price per year (one volume) is $5.00, payable in advance. Single copies cost $1.00.

Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of General Physiology*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

CONTENTS OF VOL. II, No. 1, SEPTEMBER 20, 1919

OSTERHOUT, W. J. V. Comparative studies on respiration. VII. Respiration and antagonism. Introductory note.

BROOKS, MATILDA MOLDENHAUER. Comparative studies on respiration. VIII. The respiration of *Bacillus subtilis* in relation to antagonism.

GUSTAFSON, F. G. Comparative studies on respiration. IX. The effects of antagonistic salts on the respiration of *Aspergillus niger*.

REDFIELD, ALFRED C., and BRIGHT, ELIZABETH M. The relative physiological effects of β- and γ-rays upon the egg of *Nereis*.

REDFIELD, ALFRED C., and BRIGHT, ELIZABETH M. The relative physiological effects of β-rays of different velocities.

CHAMBERS, ROBERT. Changes in protoplasmic consistency and their relation to cell division.

ZELENY, CHARLES. A change in the bar gene of *Drosophila* involving further decrease in facet number and increase in dominance.

GASKELL, J. F. Adrenalin in annelids. A contribution to the comparative study of the origin of the sympathetic and the adrenalin-secreting systems and of the vascular muscles which they regulate.

LOEB, JACQUES. Electrification of water and osmotic pressure.

Published by

The Rockefeller Institute for Medical Research

Avenue A and 66th Street, New York, N. Y.